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STUDIES ON THE RELATION BETWEEN MICROGLIA, HISTIOCYTES AND MONOCYTES *

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Ameboid phagocytic cells with the ability to store large amounts of colloidal substances are present in most of the tissues and in the blood of animals and man. In the central nervous system,[1] in the retina [1] and in ganglia [2] they are known as microglia, in other tissues as histiocytes,[3] and in the blood as monocytes.[3] A survey of the literature indicates that these three types of cells are closely related, and the studies described in this report bring further evidence of their intimate relationship.

Del Rio-Hortega [1] discovered that the phagocytic cells in the nervous system could be stained selectively with silver carbonate. In normal nervous tissue these cells exist as independent elements and are distinguished by their irregular, hyperchromatic nuclei and cytoplasmic processes on which there are thorn-like projections. Following injury to nervous tissue they increase in number, engulf cellular débris and red blood corpuscles, and fat droplets accumulate in their cytoplasm. During this phenomenon the processes become less conspicuous and the cells are transformed into round elements often called compound granular corpuscles. On the basis of the selective and distinctive staining of these cells with silver carbonate Hortega defined them as the third element of nervous tissue and named them microglia. At the present time Hortega's method is the only reliable means of their identification. The relation of microglia to histiocytes and monocytes became evident when Russell [4] demon-

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strated their ability to store trypan blue, and Lebowich [5] has recently found bacteria in microglia cells.

Costero [6, 7] cultivated ameboid cells from the brains of chickens, mice, rats, rabbits, guinea pigs and human embryos and concluded that they were microglia on the basis of their morphological properties in tissue cultures stained by Hortega's method and on their functional property of phagocytosis. He pointed out that the chief characteristics of microglia *in vitro* were the same as those manifested by histiocytes and monocytes cultivated by other workers. Wells and Carmichael [8] studied the ameboid cells of explants of the brain, periosteum and limb bud of chick embryos, using Hortega's method of staining and supravital dyes; they stated that "the striking resemblance between the cells in the brain cultures and the wandering cells in the cultures of periosteum and limb bud suggested that these two types were identical." Belezky [9] demonstrated that histiocytes in the mesenchyme of the fowl embryo are morphologically similar to the microglia seen in injured nervous tissue and maintained that histiocytes enter the nervous system along the branches of choroidal and pial blood vessels, whence they migrate into the nervous tissue and develop into microglia. Von Santha and Juba [10] found cells which they believed were primitive microglia near small blood vessels in the brain of a rat fetus weighing 15 mg. They observed that these cells first appeared in the brain near the first vascular channels and maintained that microglia are transformed "blood elements" that have migrated into the nervous tissue from the lumen of blood vessels. The finding by von Santha and Juba of microglia-like cells in the optic bulb outside of the retina and in the non-nervous tissue between the otocyst and the pharyngeal cleft is additional evidence that microglia are not peculiar to the nervous system. Subsequently, Juba [11, 12] found microglia in the brain of a human fetus 23 mm. in total length and in the brain of a fowl embryo 5 days old. Dunning and Stevenson,[13] using Hortega's method, stained cells in the normal liver, spleen and kidney of the rabbit, which, on the basis of morphological properties, reaction following injury to these organs and ability to store trypan blue, they concluded were identical with the microglia; their studies of these cells in the spleen indicated that they were histiocytes. Von Mihalik [14] recently concluded that there was no difference between the macrophages in cultures of nervous tissue, liver and subcutaneous tissue of the chicken.

The extensive literature pertaining to the nature of histiocytes has been reviewed by Aschoff,[15] Foot [16] and Maximow.[3] Illustrations of histiocytes, referred to as polyblasts, having nuclei and cytoplasmic processes similar to microglia are found in the plates accompanying the comprehensive studies of the elements of the connective tissues by Maximow [17] (Plate I, Figs. 30–35, Plate IV, Fig. 3, and Plate VIII, Fig. 5). Ranvier [18] in 1900 had already illustrated histiocytes, which he called clasmatocytes, in the omentum and mesentery of various amphibia and of mammals, fixed in osmic acid and stained with methyl violet. The nuclei of these cells are similar in shape to those of microglia, but the thorn-like projections on the cytoplasmic processes, so distinctive of microglia, are not evident. The clasmatocytes, he thought, were emigrated leukocytes *; the size and shape of their ramifications seemed to depend partly upon the elements of the tissue between which they were situated. He observed that living leukocytes put out prolongations that adhered to the surface of the warm glass chamber containing them and considered this phenomenon as evidence of the transformation of leukocytes into clasmatocytes. He also described the transformation of clasmatocytes into round cells, indistinguishable from leukocytes, following inflammatory irritation of the peritoneum.

When Aschoff and Kiyono [19] demonstrated the ability of large mononuclear leukocytes to store lithium carmine injected into the circulation, it became evident that a well known property of histiocytes is also an attribute of monocytes. In cultures of normal and pathological human lymph nodes Lewis and Webster [20] described epithelioid cells and giant cells, which they thought probably arose from or had the same origin as the large wandering cells which appeared in great numbers in almost every culture. Maximow [21] infected cultures of lymphoid tissue, omentum and connective tissue with tubercle bacilli and concluded that the epithelioid cells and giant cells arose from histiocytes ("polyblasts"). He also stated that lymphocytes transformed into "polyblasts" and sometimes acquired an epithelioid character. A "polyblast" with nucleus and cytoplasmic processes typical of a microglia cell is illustrated in his paper (Plate 1, Fig. 3). Lewis [22] reported the formation of macrophages, epithelioid cells and giant cells from mononuclear leukocytes in incu-

* The words *leukocytes* and *cellules lymphatiques* appear to be synonymous in Ranvier's article.

bated blood of the chicken, mouse, guinea pig, dog and of man. Carrel and Ebeling[23] compared cultivated histiocytes with monocytes of the adult chicken and found that "the essential properties of the blood monocyte and the tissue macrophage appear to be identical, as shown by the appearance of the colonies, their action on the medium, the mode of locomotion and the structure of the cells, their rate of growth, their food requirements and their susceptibility to certain toxic substances." The potentiality of histiocytes and monocytes to transform into epithelioid cells and giant cells has been confirmed by Hetherington[24] and Hetherington and Pierce[25]; according to their observations the lymphocytes play no rôle in the formation of macrophages, epithelioid cells and giant cells.

Material and Methods

1. *Study of Fixed Tissues*

Satisfactory preparations were obtained from the brains of chicken embryos 13, 15 and 16 days old, the liver of a chicken embryo 15 days old, peritoneal folds of a young chicken, the brain of a guinea pig embryo weighing 29 gm. and the mesentery of a mature rabbit.

The tissues were stained with silver carbonate, according to the method of del Rio-Hortega[26] for the demonstration of microglia, with slight variations.

The entire brain and liver were fixed in Cajal's formol-bromide solution:

Ammonium bromide	20 gm.
Formaldehyde (Merck)	140 cc.
Distilled water	860 cc.

The brain and liver of chicken embryos were fixed for 17 to 20 hours, but the brain of the guinea pig embryo required 41 hours of fixation. They were then heated in fresh formol-bromide to 50°–55° C. (10 minutes) and rinsed in distilled water. Frozen sections 10 to 15 microns thick were cut. They were received in distilled water, transferred to distilled water containing a few drops of strong ammonia water (a few minutes) and washed in 2 changes of distilled water. They were then placed in Hortega's strong silver carbonate solution:

Silver nitrate, 10 % (in redistilled water)	10 cc.
Sodium carbonate, 5 % (in redistilled water)	40 cc.

Strong ammonia water, just enough to dissolve the precipitate.
Solution filtered and stored in an amber glass stoppered bottle.

Sections of brain of the chicken embryos 15 and 16 days old required 30 seconds in the silver solution, but those of the younger chicken embryo and of the guinea pig embryo and the sections of embryonic chicken liver required a longer period. They were placed in one dish of silver carbonate for 2 minutes and in a second dish for 3 minutes. Silver was reduced in the sections by blowing them about forcibly in a 1 per cent solution of formalin. They were then washed in 2 changes of distilled water, fixed in 5 per cent sodium hyposulphite, washed in 2 changes of distilled water, dehydrated in 95 per cent alcohol and mounted in Canada balsam after clearing in carbol-xylol made up as follows:

Carbolic acid crystals	100 gm.
Creosote	100 cc.
Xylol	800 cc.

Fragments of peritoneum, after fixation in formol-bromide for 17 hours, without subsequent heating, were treated in the same manner as the sections of brain and liver. Requiring the longer period of staining, they were placed in one dish of silver carbonate for 2 minutes and in a second dish for 3 minutes.

2. *Study of Cultures of Tissues and Blood*

Satisfactory cultures were obtained from cerebral hemispheres of chicken embryos 13, 14, 16 and 18 days old, the livers of chicken embryos 14 and 16 days old, a kidney of a chicken embryo 14 days old, a cerebral hemisphere of a guinea pig embryo weighing 29 gm., the liver of a guinea pig embryo weighing 76 gm., the blood of chickens, two young and one mature, and the blood of a young human adult (two series of cultures).

Preparation of Cultures: The tissue was placed in a dish of Tyrode solution, where it was freed from all visible blood vessels and fibrous portions. It was then transferred to a dish of fresh Tyrode solution and cut up into pieces approximately 1 mm. square. Each piece was placed on a coverslip in one drop of plasma obtained from a chicken starved 24 hours; the tissues from the guinea pig embryos were placed in the plasma of the mother, also starved 24 hours. To the plasma was added 2 drops of Tyrode solution containing 1:10 parts of extract of chicken embryos 8 days old. The coverslips, inverted over hollow-ground slides and sealed with paraffin, were incubated at 37.5° C. Subcultures were not made, nor were the cultures washed.

All of the cultures of blood were prepared by clotting the buffy coat with one drop of extract of chicken embryos after the removal of most of the plasma. The buffy coat was placed in a dish of Tyrode solution and cut up into pieces approximately 1 mm. square. After washing with fresh Tyrode solution coverslip preparations were made in the same manner as the tissue explants, using 1 drop of the donor's plasma and 2 drops of Tyrode solution containing 1:10 parts of extract of chicken embryos.

Half of the explants were placed on coverslips coated with carbon by passing them once through the flame of a burning stick of wood. The single layer of carbon particles deposited on the glass did not interfere with the growth or visibility of the cultures.

At frequent intervals some of the preparations were unsealed. One drop of physiological saline containing 1:20,000 parts of neutral red (National Aniline and Chemical Co.) was placed on the plasma clots, the cultures were resealed and examined on a warm stage.

Staining of Cultures: Most of the cultures were stained with silver carbonate. The method used was similar to that described by Wells and Carmichael,[8] who applied to tissue cultures the method of del Rio-Hortega [26] for the demonstration of microglia in fixed tissue. The coverslips with the adherent plasma clots were fixed in Cajal's formol-bromide solution (20 to 24 hours), then heated in fresh formol-bromide to 37.5° C. (1 hour), washed in distilled water (5 minutes), placed in 25 cc. of distilled water containing 2 drops of strong ammonia water (25 minutes) and washed in 2 changes of distilled water (5 minutes in each). The coverslips were placed in Hortega's weak silver carbonate solution, made up by adding 100 cc. of redistilled water to the strong solution, and heated to 37.5° C. (5 to 9 minutes). After dipping them once in distilled water, silver was reduced in the cultures by waving the coverslips to and fro in a 1 per cent solution of formalin. They were then washed in 2 changes of distilled water (5 minutes in each), fixed in 5 per cent sodium hyposulphite (5 minutes), washed in 2 changes of distilled water (5 minutes in each), dehydrated in alcohol (5 minutes in 50 per cent, 5 minutes in 70 per cent, 5 minutes in each of 2 changes of 95 per cent alcohol), cleared in carbol-xylol (5 minutes in each of 2 changes) and mounted in Canada balsam. Some of the cultures were counterstained for fat. After washing out the sodium hyposulphite from the cultures the coverslips were placed in 70 per cent alcohol (15 seconds),

stained in a saturated solution of Sudan III in 70 per cent alcohol (5 minutes), placed in 70 per cent alcohol (a few seconds), washed in distilled water (5 minutes) and mounted in gum-glycerine.

Carbon particles within cultivated cells were satisfactorily demonstrated by staining the cultures with paracarmine. The coverslips with the adherent plasma clots were fixed in 2 per cent formalin in Ringer's solution (at least 1 hour), washed in 2 changes of distilled water (5 minutes in each), placed in 70 per cent alcohol (5 minutes), stained in Mayer's paracarmine (25 minutes), placed in 2.5 per cent glacial acetic acid in 70 per cent alcohol (5 minutes), dehydrated in alcohol (5 minutes in 95 per cent, 5 minutes in each of 2 changes of absolute alcohol), cleared in 2 changes of xylol (5 minutes in each) and mounted in Canada balsam.

Experimental

1. *Study of Fixed Tissues*

Nervous Tissue

Sections of the brains of chicken and guinea pig embryos in the latter half of development, stained with silver carbonate, were studied.

In the brains of the chicken and guinea pig embryos there are many mature microglia cells with fully developed processes (Figs. 1 and 2), such as are present in greater numbers in the nervous system of animals and man after birth. In the chicken embryos there are also swollen microglia cells with fewer processes about capillaries and just beneath the pia and the ependyma. These cells correspond to the primitive forms of microglia demonstrated by Hortega [1] in the nervous system during late embryonic life and shortly after birth.

Non-Nervous Tissue

A peritoneal fold of a young chicken, the mesentery of a mature rabbit and sections of the liver of a chicken embryo 15 days old were studied. The tissues were stained with silver carbonate in the same manner as the sections of brain.

Scattered throughout the peritoneal membrane of the chicken and rabbit there are numerous isolated cells selectively stained brown. They are distinct from the faintly stained fibroblasts and mesothelial cells, between which they lie, and have irregular, hyperchromatic

nuclei and processes characteristic of microglia cells. They appear to be the "clasmatocytes" of Ranvier.[18]

Scattered throughout the liver of the chicken embryo there are many cells selectively stained brown and conspicuous against the gray-colored liver cells and connective tissue. They are found in the connective tissue about the portal veins, between the liver cells and partly or entirely within the sinusoids and do not anastomose with each other. Morphologically they are indistinguishable from the microglia of the chicken embryos. A cell with four irregular processes on which there are numerous thorn-like projections, apparently surrounded by liver cells, is illustrated in Figure 3. The majority of the microglia-like cells in the liver are swollen and have fewer processes than the one in this figure and are partly or entirely within the sinusoids. The presence of blood cells in the cytoplasm of many of them indicates their phagocytic ability.

The cell in Figure 4 is situated between liver cells, and one of its two processes extends into a sinusoid; the cell in Figure 6 is entirely within a sinusoid and lies against its wall. It is morphologically distinct from the endothelial cells of the sinusoid, the nuclei of which appear in the picture. The endothelial cells have elongated, oval, black nuclei from which gray cytoplasmic granules extend from each pole. In sections of the liver of another chicken embryo 15 days old the reticulum was distinctly stained according to a method combining the use of silver nitrate, silver carbonate and gold chloride. In these preparations the capsule of the liver and the walls of the large blood vessels appear to be composed of coarse reticulum fibers, which are continuous with very fine threads of reticulum extending along the sinusoids, but the microglia-like cells are not stained by this method which so distinctly demonstrates the reticulum.

This study indicates that in the liver of the chicken in the latter half of embryonic development there are many cells morphologically identical with the microglia. Most of them lie partly or entirely within the sinusoids and, because of their location, their morphological characteristics and their phagocytic ability, are identified as Kupffer cells. A smaller number of the microglia-like cells are extravascular and do not differ from the histiocytes of the peritoneal membrane. Von Kupffer [27] maintained that the cells which have been named after him were endothelial cells and formed a syncytium. This view has been almost uniformly accepted, but the more recent

studies of Zimmermann [28] indicate that Kupffer cells are distinct from endothelial cells and do not anastomose with each other. Zimmermann also described cells with numerous processes situated between the sinusoids and the liver cells, which he called pericytes and believed to be related to smooth muscle cells. The histiocyte illustrated in Figure 3 of our article is morphologically identical with the "pericytes" of the liver illustrated in Zimmermann's paper (Plate 28, Figs. 188 and 189). The studies of Zimmermann and our own indicate that the Kupffer cells, commonly called reticulo-endothelial cells, are distinct from endothelial cells and, unlike reticular cells, do not anastomose with each other.

2. *Study of Cultures of Tissues and Blood*

Cultures of Nervous Tissue

Cultures of the brains of chicken and guinea pig embryos in the latter half of development were studied.

Chicken Brain: During the first day of the cultures, delicate straight fibers extended into the medium from the edge of the explants. These structures were first observed *in vitro* by Harrison,[29] who identified them as neuraxones. After the first day they were obscured by two types of cells which appeared about the explants — anastomosing cells, indistinguishable from fibroblasts, and large ameboid cells. The cells resembling fibroblasts mulplied by mitosis and eventually formed a tissue about the explants. The large ameboid cells also multiplied by mitosis, displayed no tendency to anastomose with each other and migrated to the edge of the plasma clots.

During the first few days of incubation the large ameboid cells of chicken brain, in cultures stained with silver carbonate, closely resemble the microglia in normal nervous tissue, except for swelling of their cytoplasm and the presence of pseudopodia (Figs. 7, 13 and 14). They have hyperchromatic, irregular nuclei and cytoplasmic processes on which there are thorn-like projections. As the cells migrated away from the explants, fat droplets accumulated in their cytoplasm, their processes became swollen, shorter and fewer, and they became round (Fig. 24). Such cells, often called compound granular corpuscles, are found in great numbers in areas of necrotic nervous tissue. When the large ameboid cells reached the edge of the plasma clots they flattened out against the coverslips and assumed epi-

thelioid forms. In some cultures these epithelioid forms adhered in great numbers to the coverslip over the explant. They were predominant during a late period of the cultures, usually during the second and third week and, although they were often closely packed together, cell outlines could be distinguished. Occasionally they appeared on the coverslips near the explant during an early period, in one culture as early as the third day. Epithelioid cells in cultures stained with silver carbonate (Fig. 9) are large and flat, circular in contour when isolated, and somewhat square when packed closely together. The cytoplasm of some is drawn out into a tapering sharp process. There are few granules in the peripheral portion of the cytoplasm and it is difficult to define the limits of the cells. Centrally, numerous, brown cytoplasmic granules form a conspicuous area at the margin of which there is usually one nucleus, occasionally two. The nuclei of the epithelioid cells are large, oval or round and pale, containing many fine brown granules and a few larger black granules. Fat vacuoles are usually present in the cytoplasm and are situated within or at the periphery of the central area.

The following attributes of the large ameboid cells of the chicken brain were manifest in the living preparations. The projection of pseudopods from any portion of the cytoplasm was common to all forms. A thorn-like projection on one process of the cell in Figure 14 forms the basis of three pseudopods. At the edge of the cytoplasm of the epithelioid forms there was continuous movement of small pseudopods. The large ameboid cells stored neutral red in abundance. In the forms with processes and in the round forms the dye appeared as distinct red granules evenly distributed throughout the cytoplasm. In the epithelioid forms it was confined to the central cytoplasmic area, where it appeared as a circular patch of red granules or, in an occasional cell, as a rosette about a clear, central spot (centriole). In the cultures planted on smoked coverslips carbon particles were found only in the various forms of large ameboid cells. In the forms with processes and in the round forms the carbon particles were scattered throughout the cytoplasm, but in the epithelioid forms they were segregated in the central cytoplasmic area where, in many cells, they formed a rosette (Fig. 25). The large ameboid cells appeared to be very sticky; they adhered tenaciously to the coverslips and to fibers accidentally incorporated in the plasma clots. Costero [7] demonstrated the ability of cultivated microglia cells to

cling to threads introduced into the plasma clots. After the epithelioid forms became predominant the large ameboid cells rapidly died. They were last seen alive 5 weeks after incubation.

Guinea Pig Brain: During the first two days of the cultures only neuraxones and cells resembling fibroblasts appeared about the explants. The neuraxones grew more luxuriantly than in the cultures of chicken brain. Large ameboid cells were first seen on the third day and, less numerous than in the cultures of chicken brains, they never migrated as far as the edge of the plasma clots, although they survived *in vitro* for approximately the same length of time. During their life in the cultures they exhibited all of the morphological and functional properties of the large ameboid cells of chicken brain.

Cultures of Non-Nervous Tissue

Cultures of liver and kidney of chicken embryos and of the liver of a guinea pig embryo in the latter half of development were studied.

Chicken Liver: During the first day of incubation fibroblasts appeared about the explants. They were more abundant than the similar cells in the brain cultures, multiplied by mitosis and eventually formed a tissue about the explants. A considerable number of small ameboid cells also appeared about the explants on the first day. Identified in stained preparations as polymorphonuclear leukocytes, they migrated far out into the medium and died during the first week of incubation. During the second day large ameboid cells which moved more slowly than the smaller ones appeared about the explants. They multiplied by mitosis and migrated to the edge of the plasma clots.

During the first few days of incubation the large ameboid cells of chicken liver, in cultures stained with silver carbonate (Figs. 15 and 16), are indistinguishable from the large ameboid cells which appeared early in the brain cultures. They have irregular, hyperchromatic nuclei and cytoplasmic processes on which there are thorn-like projections. As they migrated farther away from the explants fat droplets accumulated in their cytoplasm, their processes became swollen, shorter and fewer, and they became round. During the second and third week those at the edge of the plasma clots flattened out against the coverslips and assumed epithelioid forms identical with the corresponding forms in the brain cultures.

The large ameboid cells in the living cultures of chicken liver ex-

hibited all of the attributes of the living, large ameboid cells of the brain. They remained alive in the cultures for approximately the same length of time, engulfed carbon particles, which appeared as a rosette in many of the epithelioid forms, and adhered to the coverslips and to foreign fibers in the plasma clots. The ability of cultivated Kupffer cells to stick to the fibers of lens paper has recently been demonstrated by Rous and Beard.[30] As in the brain cultures, neutral red rosettes were observed only in an occasional epithelioid form, never in the round forms and in the forms with processes, in which the dye was always scattered throughout the cytoplasm.

Chicken Kidney: The cultures did not differ from those of chicken liver, except that few or no polymorphonuclear leukocytes appeared about the explants. The large ameboid cells exhibited all of the properties of the large ameboid cells in the liver cultures.

Guinea Pig Liver: During the first day of incubation small and large ameboid cells began to migrate from the explants. The small cells were identified in stained preparations as polymorphonuclear leukocytes. They were about as numerous as those in the cultures of chicken liver, migrated far out into the medium and ceased to move after the first week. The large ameboid cells moved more slowly than the small ones, multiplied by mitosis and were last seen alive 6 weeks after incubation. They were less numerous than in the cultures of chicken tissues and never migrated as far as the edge of the plasma clots.

In cultures of guinea pig liver stained with silver carbonate during the first few days of incubation the large ameboid cells resemble microglia (Fig. 8). They have irregular, hyperchromatic nuclei and cytoplasmic processes on which there are thorn-like projections. Fat droplets accumulated in their cytoplasm and the cells became round. After the third week epithelioid forms appeared on the surface of the coverslips over the explants.

The large ameboid cells of the guinea pig liver in the living cultures exhibited the following properties. They adhered to the coverslips and to foreign fibers in the plasma clots and stored neutral red in the same manner as the corresponding cells in the cultures described above. They engulfed carbon particles. In one culture a single large ameboid cell containing carbon was observed for 6 days, and camera lucida drawings were made at frequent intervals (Fig. 10). It had several processes on the eighth day, gradually became round

and, after the eleventh day, ceased to move. The culture was fixed and stained on the thirteenth day, and no nucleus is visible in this cell. Carbon particles appeared as a rosette in some of the epithelioid forms.

Cultures of Blood

Cultures of the buffy coat of young and mature chickens and of a young human adult were studied.

Chicken Blood: Three hours after incubation the explants were surrounded by a halo, 1 mm. wide, consisting of small ameboid cells. Nine hours later the halo had expanded to 2 mm. and, near the explants, there were a few larger ameboid cells, which moved more slowly than the smaller ones. At the end of the first day the small cells had migrated to the edge of the plasma clots and the larger ones had formed an inner halo about the explants. In stained preparations most of the small cells were identified as polymorphonuclear leukocytes, but the lymphocytes could not be distinguished with certainty from the free nuclei of erythrocytes. After the first week the small cells ceased to move. The large ameboid cells multiplied by mitosis and migrated to the edge of the plasma clots.

During the first few days of incubation the large ameboid cells of chicken blood, in cultures stained with silver carbonate (Figs. 11, 17 and 18), like microglia cells, have irregular, hyperchromatic nuclei and processes with thorn-like projections. Rod-shaped cells (Fig. 17) similar to those in the cultures of chicken brain (Fig. 13) and liver (Fig. 16) are predominant. As the cells migrated away from the explants fat droplets and nuclear débris accumulated in their cytoplasm and they became round and identical with "compound granular corpuscles." Toward the end of the first week, after many of the large ameboid cells had reached the edge of the plasma clots, they flattened out against the coverslips and assumed epithelioid forms indistinguishable from those in all of the cultures described above.

The attributes of the living, large ameboid cells of chicken blood were essentially the same as those of the corresponding cells in the living cultures of tissues. They engulfed carbon particles, which appeared in many of the epithelioid forms as a rosette, and adhered to the coverslips and to extraneous fibers in the plasma clots. In the neutral red preparations the dye appeared as distinct red granules scattered throughout the cytoplasm of all of the forms with processes

and of the majority of the round forms. In approximately one-fifth of the round forms it appeared as a distinct rosette of red granules about the centriole. Neutral red rosettes were never seen in the round forms of large ameboid cells in the cultures of tissues. In the epithelioid forms the dye was confined to the central cytoplasmic area where it appeared as a circular patch of red granules or, in an occasional cell, as a rosette. After the epithelioid forms became predominant the large ameboid cells rapidly died. They were last seen alive 6 weeks after incubation.

Human Blood: Twelve hours after incubation the explants were surrounded by a halo, 2 mm. wide, consisting of ameboid cells. In a culture stained at this time the larger cells can be identified as monocytes, the smaller cells as lymphocytes, and those of intermediate size as polymorphonuclear leukocytes. They are in approximately the same proportion as in the blood stream. The first cells to die in the cultures were the polymorphonuclear leukocytes. In a preparation stained on the fourth day there is pyknosis of the nuclei and disintegration of the cytoplasm of many and, in a culture stained on the sixth day, no granulocytes are seen. The lymphocytes remained alive for a much longer period of time and could be readily identified in the living cultures. They moved about actively in the medium in the same manner as those in the fresh blood of guinea pigs, described by Opie [31] in 1904. The lymphocytes gradually decreased in number and were last seen actively moving on the twenty-second day. During the entire period of their existence *in vitro* they maintained their original size, and their structure, in preparations stained with silver carbonate, is that of normal lymphocytes. During the course of incubation the monocytes proliferated by mitotic division, gradually became larger and, toward the end of the first week, assumed the characteristics of the large ameboid cells in the cultures of animal blood and tissues described above.

During the second week of incubation the large ameboid cells of human blood, in preparations stained with silver carbonate, resemble microglia cells (Figs. 12, 20, 21 and 22). The predominant forms are rod-shaped (Figs. 20 and 22) and very similar to the rod-shaped forms of microglia, which are so numerous in the brains of humans with paresis (Fig. 19.) Fat droplets and nuclear débris slowly accumulated in the cytoplasm of the large ameboid cells, their processes became swollen and shorter and the cells gradually became round

(Fig. 23) and indistinguishable from "compound granular corpuscles." After the third week the majority of the large ameboid cells assumed epithelioid forms identical with those in the cultures of animal blood and tissues described above.

The living large ameboid cells of human blood exhibited all of the attributes of those of chicken blood. They engulfed carbon particles, which appeared in many of the epithelioid forms as a rosette (Fig. 26). They adhered to the coverslips and to extraneous fibers in the plasma clots and were last seen alive 6 weeks after incubation. Neutral red was stored in the same manner. In the forms with processes and in the majority of the round forms the dye was scattered throughout the cytoplasm, but in about one-fifth of the round forms it appeared as a distinct rosette. In the epithelioid forms it was confined to the central cytoplasmic area, where it appeared as a circular patch of red granules or, in an occasional cell, as a rosette.

In the cultures of human leukocytes it was possible to observe the gradual transformation of the monocytes into large ameboid cells and to identify the polymorphonuclear leukocytes and lymphocytes, which maintained their original size and morphological characteristics until they died. In the cultures of chicken blood, however, the large ameboid cells appeared about the explants very soon after incubation and their origin was not observed. The granulocytes maintained their original size and morphological characteristics until they died. Since no normal monocytes were seen in the cultures, it is evident that they transformed into large ameboid cells. The fate of the lymphocytes of the chicken could not be determined because of the uncertainty of distinguishing them in stained cultures from the free nuclei of erythrocytes.

Discussion

(1) The following observations support the assumption that the large ameboid cells in the cultures of tissues migrated out of the tissues and only a very small number, if any, arose from the blood in the vessels of the explants: (*a*) After the removal of visible blood vessels the fragments of tissue used for cultivation were washed in Tyrode solution to remove as much blood as possible. (*b*) In stained sections of the unwashed organs used for incubation there are few, if any, blood cells other than erythrocytes in the capillaries. (*c*) If the large ameboid cells were transformed monocytes derived from the

blood in the vessels of the explants, polymorphonuclear leukocytes and lymphocytes should have appeared in the cultures of tissues, as they did in all of the cultures of blood, but neither granulocytes nor lymphocytes were seen in the cultures of brain. A considerable number of granulocytes appeared in the liver cultures, but in stained preparations no lymphocytes can be identified among them. The presence of extravascular foci of myelocytes in various stages of maturation seen in the stained sections of the liver of chicken and guinea pig embryos explains the appearance of polymorphonuclear leukocytes in the liver cultures.

(2) It is often stated that fibroblasts may transform into macrophages and macrophages into fibroblasts.[32] In none of our cultures was there any evidence of this transformation. It is very common in cultures of tissues to see large ameboid cells and fibroblasts side by side, as illustrated in Figure 61 (Fischer [32]) and in Figure 8 of this paper, but in our cultures it has always been possible, both in the living preparations and in those stained with silver carbonate, to distinguish between these two types of cells throughout their existence *in vitro*. Fibroblasts were never seen in our cultures of blood. Some of the rod-shaped cells in the cultures of human blood somewhat resembled isolated fibroblasts (Figs. 12 and 20), but the constant projection of pseudopods from any portion of their cytoplasm, their ability to phagocytize carbon particles and to store large amounts of neutral red served to identify them.

(3) Von Santha and Juba [10] demonstrated that the microglia first appear in the nervous system near the first vascular channels at a very early period of embryonic development and maintained that microglia cells are transformed "blood elements" that have migrated into the nervous tissue from the lumen of blood vessels. Our observation that monocytes in a semisolid medium transformed into cells indistinguishable from microglia suggests that monocytes are the "blood elements" that transform into microglia cells.

Summary

In fixed tissues stained with silver carbonate the histiocytes in the liver of a chicken embryo and in the peritoneal membrane of a chicken and of a rabbit are morphologically identical with the microglia in the brains of chicken and guinea pig embryos.

In cultures of tissues the large ameboid cells of the brains and

livers of chicken and guinea pig embryos and of a kidney of a chicken embryo exhibited the same morphological and functional properties. During an early period of incubation they had cytoplasmic processes and nuclei typical of microglia cells. Fat droplets accumulated in their cytoplasm and the cells became round. During the latter period of their life *in vitro* they transformed into epithelioid cells. They engulfed carbon particles, stored large amounts of neutral red and survived in the cultures for approximately the same length of time.

In cultures of chicken and of human blood the monocytes transformed into large ameboid cells that exhibited all of the morphological and functional properties of the large ameboid cells in the cultures of tissues. Neutral red rosettes were more frequently observed in the large ameboid cells of the blood than in those of the tissues.

Conclusions

Microglia and histiocytes are morphologically and functionally identical and constitute a single cell type. Monocytes may transform into cells indistinguishable from this type.

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DESCRIPTION OF PLATES

PLATE 118

FIG. 1. A microglia cell near a capillary in the brain of a chicken embryo 13 days old. Silver carbonate stain. × 550.

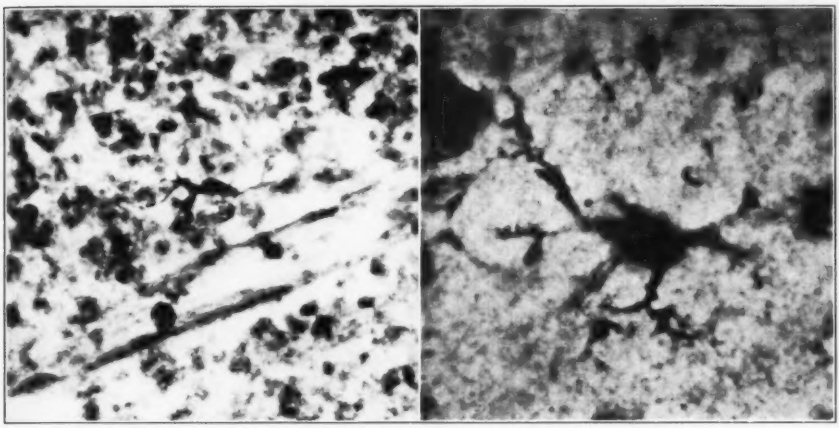
FIG. 2. A microglia cell in the brain of a guinea pig embryo weighing 29 gm. Silver carbonate stain. × 1500.

FIG. 3. A microglia-like cell in the liver of a chicken embryo 15 days old. Silver carbonate stain. × 1500.

FIG. 4. A microglia-like cell near a sinusoid in the liver of a chicken embryo 15 days old. Silver carbonate stain. Camera lucida drawing. × 2000.

FIG. 5. A central vein and radiating sinusoids in the liver of a chicken embryo 15 days old. Silver carbonate stain. × 150.

FIG. 6. A microglia-like cell (Kupffer cell) and numerous red blood corpuscles in one of the sinusoids included in the square outlined in Fig. 5. Endothelial cells line the central vein and the sinusoids. Silver carbonate stain. Camera lucida drawing. × 860.



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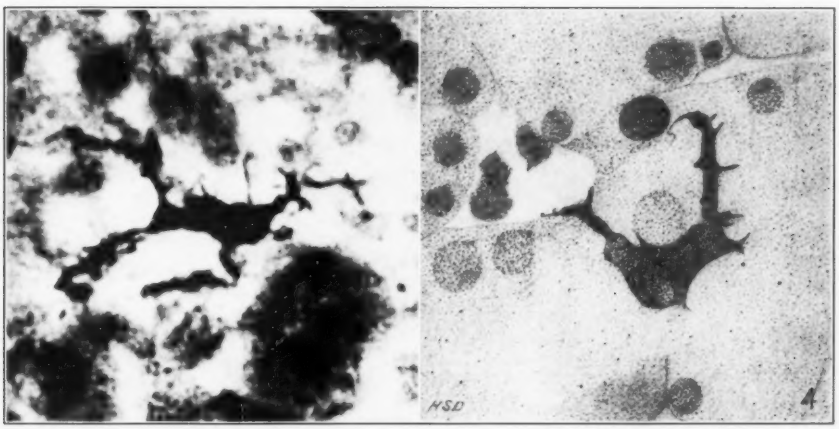

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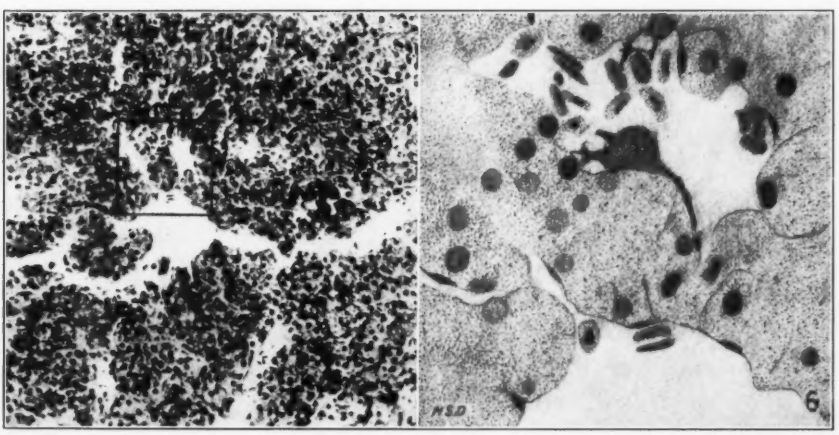

5 6

PLATE 119

FIG. 7. Large ameboid cells with processes near the explant in a 3 day old culture of the brain of a chicken embryo 18 days old. Silver carbonate stain. × 100.

FIG. 8. Large ameboid cells with processes and fibroblasts near the explant in a 4 day old culture of the liver of a guinea pig embryo weighing 76 gm. Silver carbonate stain. × 100.

FIG. 9. Epithelioid cells containing fat vacuoles in a 3 day old culture of the brain of a chicken embryo 18 days old. Silver carbonate stain. × 600.

FIG. 10. Camera lucida drawings of a single large ameboid cell containing carbon particles in a living culture of the liver of a guinea pig embryo weighing 76 gm. × 860.

FIG. 11. Large ameboid cells with processes in a 4 day old culture of the buffy coat of the blood of a mature chicken. Silver carbonate stain. × 100.

FIG. 12. Large ameboid cells with processes near the explant in a 7 day old culture of the buffy coat of the blood of a young human adult. Silver carbonate stain. × 100.

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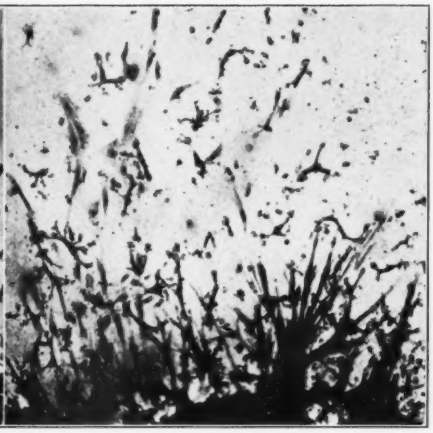

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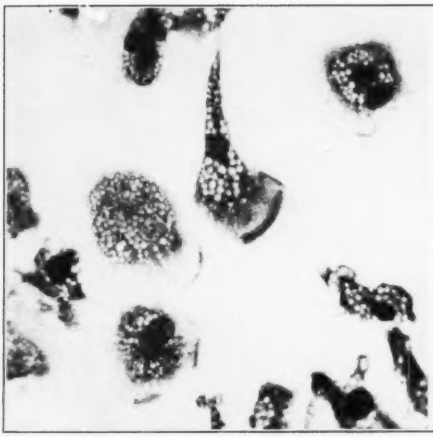

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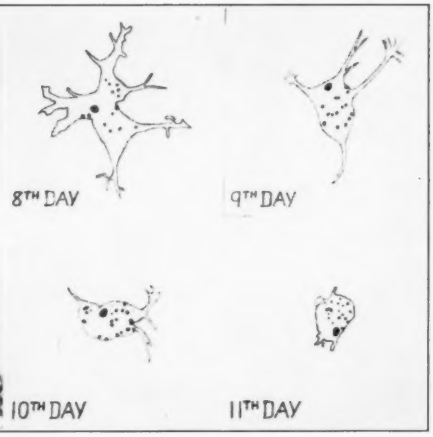

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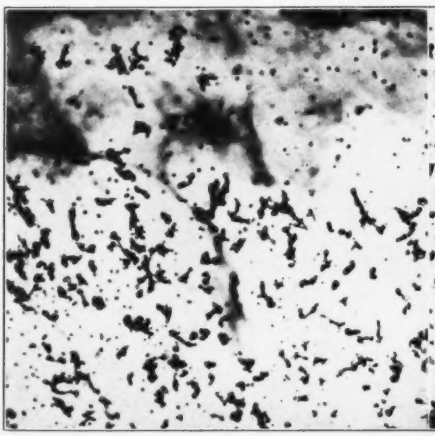

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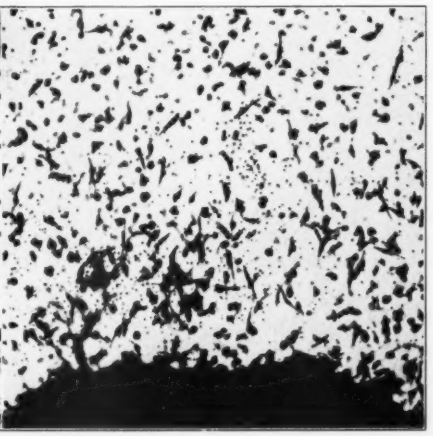


12

PLATE 120

Camera lucida drawings of stained cells, at a magnification of 860.

FIGS. 13 and 14. Large ameboid cells with processes in a 3 day old culture of the brain of a chicken embryo 18 days old. Silver carbonate stain.

FIGS. 15 and 16. Large ameboid cells with processes in cultures of the liver of a chicken embryo 16 days old. Fig. 15 is from a 4 day old culture; Fig. 16 is from a 6 day old culture. Silver carbonate stain.

FIGS. 17 and 18. Large ameboid cells with processes in cultures of the buffy coat of the blood of a young chicken. Fig. 17 is from a 2 day old culture; Fig. 18 is from a 1 day old culture. Silver carbonate stain.

FIG. 19. A rod-shaped microglia cell in a section of the brain of a human with paresis, stained with silver carbonate by Dr. Lewis Stevenson in the laboratory of del Río-Hortega, Madrid.

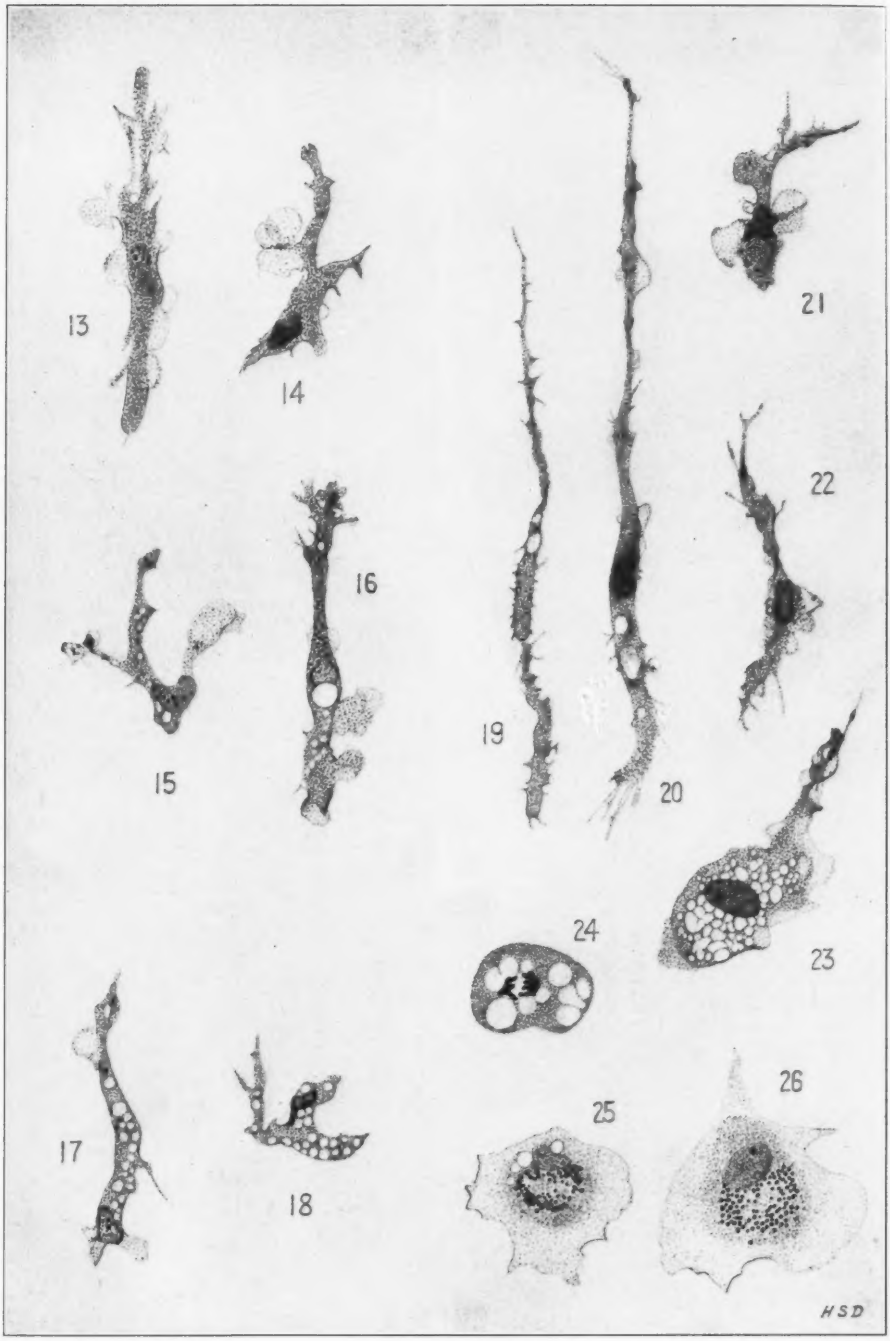
FIGS. 20, 21, 22. Large ameboid cells with processes in a 7 day old culture of the buffy coat of the blood of a young human adult. Silver carbonate stain.

FIG. 23. A large ameboid cell containing many fat droplets in a 14 day old culture of the buffy coat of the blood of a young human adult, illustrating the transition between forms with processes and round forms. Silver carbonate and Sudan III stains.

FIG. 24. A large, round ameboid cell filled with fat droplets and exhibiting a mitotic figure in a 6 day old culture of the brain of a chicken embryo 13 days old. Silver carbonate and Sudan III stains.

FIG. 25. An epithelioid cell containing a rosette of carbon particles in a 10 day old culture of the brain of a chicken embryo 16 days old. Paracarmine stain.

FIG. 26. An epithelioid cell containing a rosette of carbon particles in a 24 day old culture of the buffy coat of the blood of a young human adult. Paracarmine stain.



Dunning and Furth

Microglia, Histiocytes, Monocytes

THE CUTANEOUS GLOMUS AND ITS TUMORS—GLOMANGIOMAS *

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For centuries [44] a group of small subcutaneous tumors was recognized as the exciting point for severe and often radiating pain. In more recent times these tumors were called "angiosarcoma" in the German literature and "subcutaneous painful tubercle" in various English papers. In 1920, however, Barré [20] clearly defined their clinical manifestations and demonstrated that local excision of the tumor gives complete relief from all symptoms. It remained for Masson to establish the specific histological characteristics of these tumors and to prove their derivation from specialized arteriovenous anastomoses in the stratum reticulare of the cutis. This type of anastomosis is called the cutaneous glomus because of a close analogy to the glomus coccygeum. With their histogenesis established, various names have been given to the tumors. These include glomus tumor, tumeur du glomus neuromyo-artériel, and angiomyoneuroma. Many of these terms are cumbersome. None denotes the fact that the tumors represent an overgrowth of a specific type of arteriovenous anastomosis and form a subdivision of the group of angiomas. If the name were to call attention to this fact, the prognosis and surgical approach, as well as the histogenesis, would be indicated at once. Despite the already too numerous terms applied to the lesion, it seems worth while to suggest *glomangioma* to apply to tumors arising from the cutaneous glomus.

On account of the organoid character of glomangiomas, a description of the normal histology of the glomus will be given and the fate of its various parts will be followed in the tumors.

THE NORMAL CUTANEOUS GLOMUS

A specialized arteriovenous anastomosis, now called the cutaneous glomus, was described first by Hoyer,[7] but its histological structure was made clear by Sucquet.[14] More recently, important contribu-

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tions have been made to the knowledge of the normal glomus by Masson [11,12] and Popoff.[13]

The glomus is distributed widely but unequally over the cutaneous surface of the body and is by far most frequently encountered on the extremities. It is found most abundantly in the region of the nail bed and at the tips of the digits, while it is present in large numbers on the palmar surface of the first, second and third phalanges of the upper and lower extremities. These anastomoses are demonstrable also on the thenar and hypothenar eminences of the hand and on the sole of the foot near the heel.[11,12,13] In smaller numbers they are found on the cutaneous surfaces of the upper and lower extremities and in the corpora cavernosa penis and corpus cavernosum urethrae.[1] By a study of serial sections Popoff [13] found in the great toe of a patient 20 years of age 18 of these arteriovenous anastomoses on the ventral surface, 10 on the lateral surfaces, 24 in the nail bed and 12 in the nail matrix. Grant and Bland [4] stated that the glomus is much more abundant since they found 593 in the nail bed of the second toe and 296 in the toe pad. They, however, did not use serial sections, which may explain the discrepancy in part. It seems likely that there is considerable anatomical variation in the distribution of the glomus. The diameter of the glomus depends upon its location, those of the pad of the toe measuring 120–220 microns and those of the nail bed 60–150 microns.[13] The glomus undergoes considerable alteration with age. It is formed imperfectly at birth and reaches its maximum development only in young adulthood. It atrophies in old age even in those individuals in which it escapes arteriosclerotic changes.[12,13]

The cutaneous glomus occupies a specific zone of the cutis — the stratum reticulare. Its afferent artery arises from arterial branches in the subcutaneous tissue which pursue a course parallel to the surface of the skin. These continue upward and divide into two branches in the stratum reticulare. The larger branch bends at a right angle and continues parallel to the skin while the smaller branch divides again. Some of the divisions of the smaller branch contribute to the formation of arteriovenous anastomoses, while others go to the papillary bodies and the artery itself ends as the artery of the papillary body. The arterial branches constitute the essential parts of the glomus and have been called the Sucquet-Hoyer canals.[13] The canals pursue an S-like course and have narrow, irregular lumens. A single afferent

glomic artery forms one to four separate Sucquet-Hoyer canals. When multiple they are situated in contiguity, grouped together by fibrous tissue which, without forming a capsule, isolates them by orientation of the collagenous bundles.[11] The endothelial cells are rather large and have oval nuclei. They are placed in two or three rows without an underlying elastic lamina. In the afferent artery at the beginning of the Sucquet-Hoyer canal there are cushion-like endotheliomuscular elevations which, according to Popoff,[13] serve to direct blood flow. The endothelium is surrounded by a rather thick muscular coat in which indistinct inner longitudinal and outer circular layers have been described,[11,12] though Popoff [13] was unable to confirm this finding. Among the muscle cells are large cells with clear or vacuolar cytoplasm which does not contain stainable glycogen, fat or mucin. Their histogenesis is a fundamental problem in an understanding of the glomus since they, more than any other single structure, are peculiar to these anastomoses. These have been called "epithelioid" cells. They may be called *glomus cells* because they are found only in the cutaneous glomus, the glomus coccygeum and their homologues. The name "epithelioid" has been carried on from the time when these structures were thought to be endocrine in nature and should be abandoned. These cells have been described as postembryonal angioblasts,[8] as modified smooth muscle cells,[13] and as specialized neuromuscular cells.[11,12] Their nature, as seen in tumors arising from the glomus, will be treated in the second section of this paper. The glomus cells are in intimate association with a rich network of non-myelinated nerve fibrils which may be demonstrated either by various silver methods [13] or by Masson's trichrome stain.[11,12] These can be followed to the larger periglomic nerve trunks. Along the course of the non-myelinated nerves the round and rod-like nuclei of Schwann's sheath are found. These are encountered more frequently as the larger nerve trunks are approached. At the point at which the Sucquet-Hoyer canal begins, there is a distinct collagenous ruffle containing an elastic lamina under the endothelium.[13] The remainder of the glomus is lacking in elastic tissue, the elastic lamina being resumed at once in the vein of termination of the Sucquet-Hoyer canal. In the meshes of coarse collagenous tissue which surround the glomus there are small arterioles which, arising proximal to the Sucquet-Hoyer canal, supply the clear neuroreticular zone around the canal and the nerve trunks of the peri-

glomic zone. These preglomic arterioles do not communicate with the lumen of the Sucquet-Hoyer canal. The arterioles have thin walls with few muscular cells and no neuroreticular elements.[13] The primary collecting veins are supplied with elastic tissue but present few muscle cells. The collecting veins of the stratum reticulare and stratum subcutaneum are provided with valves, according to Popoff.[13] The primary collecting veins open into the subpapillary veins and through the latter into deeper veins.

A number of structures have been described which are homologous with the cutaneous glomus. The most important of these is the glomus coccygeum. This structure in the sacral region has been shown by von Schumacher [15] to result from a transformation in an arteriole issuing from the midportion of the sacrum. The cells of the arteriolar walls become larger, clearer, and take on an "epithelioid" appearance. They are in every respect identical with the glomus cells of the cutaneous arteriovenous anastomoses. While the glomus coccygeum appears in young embryos (145–170 mm.),[8] the cutaneous glomus is absent from the fetus and even premature infants.[13] The cutaneous glomus is also homologous with the organ of Ruffini [11] and the caudal glomeruli of animals.[15]

The cutaneous glomus is distributed widely among mammals in much the same regions as in man. Hoyer [7] described its presence in rabbits, cats and dogs, and its absence in guinea pigs. Grosser [5,6] pointed out its presence in mice and its absence in reptiles. The glomus is developed to a high degree in birds.[1] It is of particular significance that the glomus has not been described in cold blooded animals and that it is so prominent in birds, whose body temperature in general is above that of mammals.

The function of the glomus was understood very imperfectly until the work of Lewis and Pickering,[10] and Grant and Bland,[3,4] demonstrated its importance in temperature regulation. They showed that the glomus serves for the maintenance of the temperature of exposed parts and the regulation of loss of heat. By direct observation of the rabbit's ear, they found it difficult to see any anastomoses when a rabbit is kept warm. The anastomoses dilate readily on gentle mechanical stimulation. Even slight stimulation of an area with a rounded glass rod causes local dilatation of vessels and of the anastomoses arising from them. The anastomoses dilate when the nearby skin is pricked. Acetylcholine and adrenalin cause local dilatation of

the arteriovenous anastomoses which is independent of the nervous system. Histamine acts in the same manner as mechanical stimulation. The anastomoses react to nervous stimuli and with weak currents may do so independently of the afferent artery. On contact they dilate vigorously and quickly. To thermal stimuli the reaction is also striking. The ear may be raised to 35 or 40 degrees C. before any great dilatation occurs. However, when the surrounding temperature is lowered below a somewhat variable point (usually about 15 degrees C.), the anastomoses open although other vessels are at first constricted. If the cooling is slight or brief only the anastomoses open. When the animal is warmed again the anastomoses return to normal before the vessels. Grant [3] stated that it is mainly through the agency of the glomus that the temperature of the rabbit's ear is maintained when it is exposed to cold. He concluded also that the glomus is an important factor in regulating body temperature of the rabbit, aiding the dispersal of heat by allowing an enormous blood flow through the ear.

Lewis and Pickering [10] showed that in raising the temperature of the room in which the subject is sitting, vasodilatation is produced in the limb in part by a direct effect of temperature on the vessels, and in part by an effect through the central nervous system. The tips of the fingers are usually coldest when the body as a whole is cold, but this warms most rapidly because of the opening of the arteriovenous anastomoses, which are most numerous in the nail bed and finger tips. The blueness of the nail beds in persons exposed to temperatures slightly below those to which they are accustomed is a common observation. In addition, Clara [1] believed those in the penis are associated with the mechanism of erection of that organ.

It is also possible that the cutaneous glomus acts as a shunt in the maintenance of blood pressure. By increasing the passage of blood through the anastomoses the cutaneous capillary bed would be decreased. However, neither Popoff's anatomical studies [13] nor the physiological observations mentioned above prove this point.

In the present study the pad of the great toe was examined in a comparatively small number of individuals in sections both perpendicular to and parallel with the surface. These were stained with Mallory's phosphotungstic acid hematoxylin or occasionally with hematoxylin-eosin. The arteriovenous anastomoses corresponded in general to the descriptions of Masson [11,12] and Popoff.[13] No constant

association of the glomus with such cutaneous structures as sebaceous glands, hair follicles or tactile corpuscles was noted. However, medium sized nerve trunks were found in the periglomic zone adjacent to the outer layers of glomus cells in nearly all cases. Valves were seen in a few veins of the stratum reticulare in the sections, confirming the observation of Popoff.[13]

The pathological changes of the glomus in inflammation, arteriosclerotic gangrene, diabetic gangrene, thrombo-angitis obliterans and supernumerary digits were studied by Popoff,[13] and those in syringomyelia and old age by Masson.[11]

Glomangiomas

In 1924 Masson [33] showed for the first time that certain small cutaneous tumors represent organoid overgrowths of the cutaneous glomus. In the course of the 11 following years, various pathologists confirmed Masson's findings, so that 58 instances of glomus tumor were recorded in the literature. These were obtained from 56 patients, Adair [17] describing a patient presenting 3 glomus tumors of the forearm. The present material consisted of seven lesions, bringing the total number of glomus tumors recorded as such to 65. However, it is possible to find in the earlier literature many descriptions of lesions associated with the syndrome now known to be caused by tumors of the cutaneous glomus. A group of such cases was collected by Chandelux [44] under the name of "subcutaneous painful tubercle." He pointed out a relation to tactile corpuscles, the presence of numerous dilated blood vessels and the formation of a pseudo-erectile vascular bed. Greig [26] reviewed the English literature in this regard and found 20 cases, to which he added 3 of his own, described usually as "painful subcutaneous nodule" but probably representing tumors of the glomus. Kolaczek [45] in 1878 presented a group of cases under the name of angiosarcoma. Some of these were subungual and produced the symptoms of glomus tumors. Nine years later, Kraske [46] followed Kolaczek in describing a very painful subungual tumor of the left middle finger as an angiosarcoma. He pointed out that the lack of invasiveness and complete relief by local excision made classification of such tumors in the group of sarcomas somewhat doubtful. As late as 1927, Carstensen [43] described an "angiosarcoma" beneath the nail of the right ring finger. The symptoms and microscopic findings were very similar to those

TABLE I

Data on Seven Cases of Glomangioma

Case No.	Age	Location	Symptoms	Relation to trauma	Duration	Gross appearance	Relief by local excision	Adjacent glomus	Nerve trunks in surrounding connective tissue
	yrs.				*yrs.*				
1.....	48	Left upper arm	Local pain on pressure and spontaneously. Radiation of pain to shoulder, left pectoral region and occiput. Described in detail in text	Followed blow on arm	20	Firm, dark red nodule 0.3 cm. in diameter	Immediate and permanent	One in adjacent tissue	Present
2.....	52	Right thigh near groin	Severe local pain on pressure	None	20	Bluish nodule 0.7 cm. in diameter	Immediate and permanent	None	Present
3.....	50	Subungual finger	Local pain on pressure	None	4	Bluish nodule 1 x 0.5 cm.	Immediate and permanent	None	Present
4.....	74	Dorsum of right forearm	Severe and increasing pain, spontaneous or elicited by pressure	None	Many; severe symptoms for 1 year	Elevated, purplish gray tumor 0.7 x 0.5 cm.	Immediate and permanent	None	Present
5.....	79	Posterior aspect right arm 5 cm. above elbow	Local pain on pressure. Contact of clothing sufficient to stimulate pain	None	40	Bluish nodule 0.7 cm. in diameter	Immediate and permanent	None	Present
6.....	57	Lateral chest wall 6 cm. below right axillary pit and 4 cm. posterior to lateral border of pectoralis major muscle	Intermittent spontaneous pain. Pain also elicited by slight pressure	None	18	Soft reddish mass 1 x 0.4 x 0.6 cm.	Immediate and permanent	One in adjacent tissue	Present
7.....	42	Subungual left ring finger	Neuralgic type of pain starting from region of tumor and radiating up arm. Pain elicited by cold or pressure	None	9	Reddish gray nodule 0.5 x 0.4 cm.	Immediate; operation recent	None	Present

of glomus tumors. These are only a few of the references to this type of lesion in the older literature. Because of the lack of complete clinical and histological data in many cases of this type, evaluation of them is difficult and serves no useful purpose. This group emphasizes, however, that the condition is considerably more common than was supposed.

The material upon which the present study was based consisted of 7 specimens, all of which were removed surgically. Blocks from each were fixed promptly in Zenker's fluid. Additional blocks of the tumors in Cases 2, 3 and 7 (Table I) were also fixed in 10 per cent neutral formalin. The material in Zenker's fluid had been used for routine studies but all available fragments were embedded in paraffin and cut in serial sections. Ribbons of three to five sections were placed on slides and stained successively with hematoxylin-eosin, eosin-methylene blue, Mallory's phosphotungstic acid hematoxylin, Foot's modification of Hortega's silver carbonate method for reticulum, Mallory's aniline blue-acid fuchsin-orange G connective tissue stain, Weigert's resorcin-fuchsin elastic tissue stain, and Van Gieson's hematoxylin- picric acid- acid fuchsin. The formalin-fixed material was embedded in celloidin and stained by Cajal's reduced silver method for nerve fibers and Bielschowsky's method for peripheral nerve fibers.

The gross appearance of glomus tumors is rather characteristic. They are always small, those in this series being 1 cm. or less in diameter, while the largest described in the literature is less than 3 cm. in diameter. The tumors vary in color from deep red through shades of purple to blue, the last color being perhaps the most common. The surface is covered by a layer of skin or nail without ulceration or erosion, at least in the 7 instances in the present series. Sharp demarcation from the surrounding tissues is present. The cut surface exudes blood as it is exposed and presents a grayish tint when the blood has been released. Occasionally, as in Case 1, there is a large vessel near the tumor and connected with it.

Upon histological examination, the tumor is found to be composed of contorted vessels with certain peculiarities of their walls which are seen under normal conditions only in the cutaneous glomus and its homologues. Despite considerable variation from case to case and even in the same tumor, as followed in serial sections, vessels can be found which resemble very closely the Sucquet-Hoyer canals of the

normal glomus. As described by Masson,[34] and Barré and Masson,[22] the vessels of glomus tumors are of two types. In the first of these there are one or two layers of smooth muscle in circular arrangement which are separated from the endothelium by a collagenous membrane. These smooth muscle cells are surrounded in a circular manner by shorter, larger cells with clear or pale staining cytoplasm and a compact globular nucleus. These blend with polygonal glomus ("epithelioid") cells. The second type of vessel presents endothelium bordered by glomus cells without the interposition of smooth muscle fibers. These vessels correspond more nearly to those of the normal Sucquet-Hoyer canal than the previous ones. In the 7 cases of the present series vessels of both types were found in all the tumors, though the relative proportions varied considerably from tumor to tumor and from section to section. Cases 1, 4 and 7 for example, presented only very few vessels of the first type and they might have been missed had the tumor not been examined in serial sections. Some carefully studied cases, however, are composed apparently of but one type of vessel. Mason and Weil [32] stated that all the vessels in their tumor were of the second type. As described by Masson [34] and as followed in the present material, vessels of the first type are directly continuous with those of the second.

The vessels of glomangiomas usually lack any semblance of elastic laminae. Occasionally, vessels within a glomangioma show well defined elastic layers. These, however, have only smooth muscle cells of the usual type in their walls and do not assume the characteristics of glomic vessels. They may, therefore, be regarded as vessels included in the growth of the tumor or as the preglomic arterioles supplying the glomus from which it arose. The absence of elastic laminae furnishes additional evidence for the derivation of glomangiomas from the Sucquet-Hoyer canals because these canals are lacking in elastic tissue while the preglomic arterioles and postglomic venules are provided with it.

Since none of the glomangiomas in this series showed ulceration, infiltration with any considerable number of inflammatory cells was not encountered. From place to place, basophilic leukocytes were found in rare instances while a few hemosiderin-laden phagocytes were seen occasionally.

About these tumors there are dense collagen fibers, blending with those of the surrounding connective tissue. Usually they resemble

fibers pushed aside in the centrifugal growth of the tumor rather than those of a true capsule. They represent an increase in amount of connective tissue over that seen about the cutaneous glomus normally and the layer is wider only because of the greater size of the contained structure. This is a further demonstration of the lack of tissue destruction caused by glomangiomas.

There is a type of cell which is peculiar to the cutaneous glomus, its homologues and glomangiomas. For this reason, as discussed previously, it should be called the "glomus" cell and not the "epithelioid" cell. Such cells are round or polygonal in shape. The cytoplasm is clear or pale staining but does not contain glycogen, fat or mucin. In the most carefully preserved Zenker-fixed material in the tumors of this series the cytoplasm takes a faint, homogeneous acid stain. There are no external or internal myofibrils. A few of the cells contain small granules, present after both Zenker and formalin fixation and in sections embedded in celloidin and paraffin. They stain deeply with basic dyes and are brought out clearly as black dots in the Cajal reduced silver preparations. The cytoplasm is brownish after fixation in chrome salts. In outline and in nuclear details these cells correspond to those adjacent to them. The appearance may represent an early degenerative change. The nuclei of all cells contain a large amount of chromatin which is arranged in a finely granular mat without the formation of a network. Mitoses are absent. These cells may be arranged in three ways. They may be closely packed together in the walls of vessels with single coarse collagen fibers intervening between adjacent cells. When arranged in this fashion there are groups of two or three nuclei at rare intervals which are separated by homogeneous cytoplasm without definite cell boundaries being demonstrable either with Mallory's aniline blue connective tissue stain or with Foot's modification of Hortega's silver carbonate method for reticulum. The cells also may be clumped in large masses without vascular lumens. In the third arrangement the cells are separated from one another by a homogeneous material which takes the same stains as collagen but somewhat lighter than the collagen fibers. In it the glomus cells are teased away from the larger masses. It is then that their outline is seen best and the delicate collagen fibers and nerve filaments are studied to particular advantage. Because of its intimate association with the glomus cells, it probably represents material produced by their degeneration.

This material is not found in the normal glomus though it seems rather characteristic of glomangiomas. These different arrangements of glomus cells result in great variability of general appearance in glomangiomas. Solid masses of cells alternate with areas in which cells are few, and homogeneous intercellular material is abundant. In still other portions, vascular lumens are so numerous and large that the tumors take on an appearance similar to that of the more common cavernous hemangioma. Yet even here close inspection shows that the cells making up the walls of the vessels are glomus cells and not the elongate smooth muscle cells of the hemangioma. In the cavernous portions of glomangiomas numerous nerve fibers are demonstrable with Mallory's phosphotungstic acid hematoxylin stain.

Among the glomus cells run large numbers of nerve trunks, mostly non-myelinated. At the periphery of the tumors the trunks are large and present a prominent Schwannian sheath. This is lost as the filaments ramify among the glomus cells, the nuclei becoming fewer the farther the nerve filament is followed from the periglomic trunks. The nerve fibers branch and terminate around glomus cells by the interposition of nerve endings. A nerve ending was illustrated by Mason and Weil.[32] In the very fresh and carefully fixed material of Case 7 Cajal's reduced silver method shows that such nerve endings are more numerous. With the usual perversity of silver methods there are many areas in which no endings are seen but enough are impregnated to justify the statement that such is the method of termination. The nerve endings are composed of two or three minute filaments spread out over the glomus cells. They frequently have small nodosities or bulbous enlargements along their course, especially at their extremities. Sections stained with aniline dyes show the same rich plexus of nerves but give no indication of the method of junction of nerve and glomus cell. Masson[33,34] stated that there is a syncytial relation between the two, a finding not confirmed in this material. Studies with Mallory's aniline blue connective tissue stain, Cajal's reduced silver method, and Masson's trichrome stain have given no support to the older views that the glomus cell is endocrine or angioblastic in nature.[8]

By choice of fields for study from different tumors and at different levels it is possible to follow the process of development of the various elements found in glomangiomas. The process of differ-

entiation is two-fold. Nearest a vascular lumen the cells are elongate; as one progresses centrifugally these become round in outline and lose their external and internal myofibrils. In areas without vascular lumens the elongate cells are at the center and the round ones at the periphery. The cytoplasm becomes homogeneous. The nuclei also become rounded and the chromatin arranged in a finely granular mat. At the same time the argyrophile reticulum investing the cells becomes coarser and is brought out intensely with aniline collagen stains. The second factor in differentiation is that of arrangement of the nerve fibers. Bundles of nerve fibers are gathered in large periglomic nerve trunks. These traverse the adjacent connective tissue and turn sharply to be incorporated in the tumors. Like the change in the smooth muscle cells, the nervous connections are established first at the periphery and development progresses toward the center.

The process of formation of the atypical Sucquet-Hoyer canals of glomangiomas consists of these two processes which go on simultaneously and which seem to be interdependent. In this way the two types of vessels described by Masson [33,34] represent different stages of development of Sucquet-Hoyer canals. In the second type the process of differentiation has reached the lumen, while in the first type layers of elongate smooth muscle cells still remain.

The glomangiomas may be considered an overgrowth of a specific arteriovenous anastomosis and the neurones terminating in it. They thus represent proliferation of mesodermal and ectodermal elements. Masson [47] showed that pigmented nevi represent the proliferation of an entire end organ, in the dermis consisting of Meissner's corpuscles and chromatophores, and in the epidermis of Merkel-Ranvier corpuscles and chromatophores. A close analogy can be drawn, then, between glomangiomas and pigmented nevi. The comparison is exact only if one considers the chromatophores mesodermal. If subsequent investigations establish their ectodermal derivation then the nevi represent proliferation of tissue derived from only one germ layer.

The clinical syndrome produced by these tumors is as characteristic as their histological appearance. A summary of the clinical data of each case is given in Table I. The symptoms caused by each of the tumors were strikingly similar. Hence, the following case (Case 1) is described in detail as representative of the entire group.

Report of Case

Clinical History: A previously healthy barber, 48 years of age, was admitted to the hospital for removal of a painful tumor of the left upper arm. Twenty years before the patient sustained an injury to the outer side of the left upper arm at the deltoid insertion during a barroom scuffle. There was only slight immediate discomfort. However, for several weeks a spot could be noted at the site of the injury which, as time went on, changed color from red to greenish yellow to blue. The soreness about the area never entirely went away and after several months the region again became acutely painful. During the entire interval of 20 years a slight touch on this bluish nodule elicited localized sharp pain. If the patient did hard work with his left arm, particularly when the arm was held over his head, pain would be experienced about the anterior and posterior aspects of the shoulder, in the left pectoral region and on the left side of the neck. From the latter site it radiated toward the occiput. At such times the nodule became deeper blue and somewhat larger. He was able to sleep only if he placed his arm across his chest to prevent pressure on the nodule. For the two years preceding his admission he was unable to bathe his arm with any degree of energy. The patient found it necessary to protect his left upper arm when in a crowd because of the severe pain caused by inadvertent pressure. He worried a great deal about the condition. There was a loss of weight of 11 pounds in the 2 years. With the exception of the tumor, the general physical examination, including the neurological findings and roentgenograms, were normal. The tumor presented itself as a bluish area 3 mm. in diameter just above and anterior to the insertion of the left deltoid muscle. It showed little induration though the electrifying pain caused by palpitation made the examination unsatisfactory. The bluish area was removed with the patient anesthetized by nitrous oxide-oxygen. The operation was followed by immediate and permanent relief of pain.

The tumors are located usually upon the extremities, only 2 examples having been encountered elsewhere (Table II). One of these occurred over the acromion; the other, Case 6 of this series, was found on the lateral chest wall. Twenty-one of the 65 examples tabulated were located in the nail bed, 20 of them on fingers, 1 on a toe. This is a most unusual site for tumors, though Adair, Pack and Nicholson [18] have pointed out several other types of tumors in that location. Most interesting of these is the melanoma, which shows great malignancy in this location, in contrast to the benignancy of glomangiomas.

The most characteristic clinical symptom of glomangiomas is pain. This may be spontaneous and intermittent or may arise only when the tumor is touched. The pain has a stabbing, burning character, described by one patient (Case 6) as that produced by a "red hot poker." It may often be elicited by the slightest pressure, as of clothing or of bedcovers. Patients develop curious habits associated with the protection of the tumors, such as carrying the hand in the

pocket to keep the trousers from touching a tumor on the thigh. A patient in this series (Case 6) for many years gave a history of awaking periodically from his sleep because of the pain. He would then get up and pace the floor, meanwhile holding his hand over the tumor so that his nightclothes did not touch it. The pain may also radiate over the extremity, shooting out from the tumor as a focal point. At times patients experience severe neuralgic pain but are entirely un-

TABLE II

Distribution of Glomangiomas

Lower Extremities		18
Thigh	9	
Region of knee	2	
Leg	3	
Foot	2	
Sole	1	
Subungual	1	
Location on extremity not given	2	
Upper Extremities		44
Upper arm	4	
Forearm	10	
Hand	30	
Thenar eminence	1	
Palmar surface of finger	1	
Dorsal surface of finger	1	
Palmar fascia	1	
Subungual	20	
Location on hand not given	6	
Acromion		1
Chest Wall		1
Location not given		1

aware of the tumor as an inciting point until on careful physical examination it is discovered (Case 7).

The tumors may be associated with sympathetic disturbances of vasomotor character, such as a Claude Bernard-Horner syndrome, as in Barré's [20] early case. He stated in the discussion of his original paper [20] that excision of the subungual tumor of the finger was followed in several days by the disappearance of the Claude Bernard-Horner syndrome.

Several cases have been described in which pain was produced when the patient went from a warm room to the cold outdoors. One patient (Case 7) suffered more severely when the finger bearing the tumor became cold than when pressure was made upon the tumor. Sometimes the pain has been relieved by immersion of the finger in

hot water.[28] One instance[37] showed a measurable difference in cutaneous temperature between the extremity bearing the glomus tumor and its fellow. Sympathetic disturbances originating in these tumors may have an unexpectedly wide distribution. The tumors may become more blue when painful than in the asymptomatic interval.

Let us now consider the mechanism of production of pain. Masson[33] believed it to be caused by pressure on adjacent pacinian corpuscles. This view has been generally accepted. Evidence can be gathered that the pain is associated with the dilatation of the glomic vessels. The increased blueness during paroxysms of pain is one point in favor of this view. At times, hard exercise with the extremity bearing the tumor (Case 1) causes pain, which might be explained by the increase in volume of blood passing through all vessels of the extremity. In consideration of the normal physiological reactions of the glomus it has been shown[10,4] that when the temperature of the extremities is lowered, the glomic vessels are the first to dilate and may be the only ones to do so. Hence, cold would cause dilatation of the glomic tumor vessels. Those of the finger tips and distal phalanges are the first to respond,[10] which fact might be correlated with the presence of some of the most painful of the glomus tumors in the nail beds. Normal glomic vessels dilate in response to trifling tactile or chemical stimulation of the cutaneous surface in the experimental animal.[3] The dilatation of the vessels of glomus tumors, then, is associated with the paroxysms of pain. Moreover, it has been shown that there are many nerve fibers which enter the vessel walls and terminate about the glomus cells with nerve endings closely applied to their margins. The dilatation of the vessels would then set up impulses which would be carried to the large periglomic nerve trunks in the connective tissue surrounding the tumor. Further dissemination along these pathways might explain some of the more distant sympathetic disturbances which are relieved by the removal of glomangiomas. In this respect the glomangiomas behave functionally as overgrowths of neurovascular end organs, corresponding to their histological characteristics as such. That this radiating type of pain is only an exaggeration of the normal is shown by its close resemblance to that caused by prolonged immersion of the hand in ice water. The sensation of pain is not limited to the part of the hand in the water but extends to the elbow or even the shoulder in this

normal reaction to a strong stimulus just as it does in patients with a glomangioma, but in the latter case a much weaker stimulus will elicit the response.

The relation of a large number of glomangiomas to trauma is striking. In nearly half the cases some single severe injury is followed directly by the development of a glomus tumor. In Case 1 of this series a blow during a barroom scuffle was followed by a hematoma which gradually faded but never entirely disappeared, the bluish nodule slowly taking its place. Many similar instances are recorded as of a blow on the fingernail from the top of a desk [33] or injury to the knee in a bicycle accident.[32] Numerous other types of trauma have been followed in the same manner by glomangiomas.[40,31]

Since pain is a symptom which brings a tardy patient to the physician, it might be expected that treatment would be sought promptly. As a matter of fact, quite the contrary is the case. Of those examples in the literature in which the length of time from the onset of symptoms was stated (including the present series), the average duration was 14 years. In seeking an explanation we find that the symptoms increase slowly over many years. Perhaps the patients accustom themselves to the knife-like pains and pay little attention because of their familiarity. Furthermore, some patients do not recognize the tumor as a focal point for the pain, but believe that they suffer from "neuralgia" or similar malady. That such beliefs are not restricted to patients is shown by the fact that in one instance a periarterial sympathectomy was performed for the intractable pain with only temporary relief.[33] Some patients, also (Cases 6 and 7 of this series and that of Prodanoff [39]) have been considered psychoneurotics and have been turned away without treatment until the tumor was recognized at a later date.

Local surgical excision produces complete and immediate relief from symptoms. The only instance in which there was any residual pain was that of Lortat-Jacob and Brosse,[29] reported shortly after operation. Local anesthesia is usually used in removal of glomangiomas. Interestingly enough a very large quantity may be required [17] if local infiltration is done. Nerve block is very satisfactory and anesthesia by this method is as easily obtained as in other lesions of the same areas. Irradiation is without avail.[17]

Glomangiomas are entirely benign. No instance of recurrence has

been recorded. They are not invasive and are easily removed entire. This is of especial importance because extensive operations were often done when the nature of the lesion was not recognized, especially when a diagnosis of angiosarcoma was made. The immediate and permanent relief from intractable pain of many years duration makes these patients among the most grateful in the practice of surgery.

Summary

The cutaneous glomus is an arteriovenous anastomosis in the stratum reticulare of the cutis, which is homologous with the glomus coccygeum and several less important vascular structures. These have in common a specialized glomus cell, which is a modified smooth muscle cell with abundant nervous connections. The cutaneous glomus has an important function as an arteriovenous shunt in maintaining the body temperature and perhaps the blood pressure.

From the cutaneous glomus, tumors arise which form a subgroup of the hemangioma. The term *glomangioma* is suggested for them to indicate their derivation and character.

Glomangiomas appear as small bluish nodules on the extremities or adjacent portions of the shoulder girdle. Very frequently they are located in the nail bed. Microscopically the tumors are composed of cells identical with those in the walls of the normal cutaneous glomus and its homologues. Nerve trunks are numerous in the connective tissue about the tumors and nerve filaments pass among the glomus cells in large numbers. Occasionally elongate smooth muscle cells are seen either in solid masses or adjacent to vascular lumens.

The glomangiomas represent the overgrowth of the entire arteriovenous anastomosis and in doing so their cells show a two-fold differentiation. The elongate smooth muscle cells lose all myofibrils, while the reticulum investing them becomes much coarser and stains intensely with collagen stains. Secondly, the periglomic nerves grow into the tumors and their terminal filaments end about the differentiating smooth muscle cells with the interposition of nerve endings. These two processes result in the formation of the glomus cells and are apparently interdependent.

The tumors are associated clinically with severe radiating pain of neuralgic type. In character and distribution this has many similarities to the response of the normal glomus to much greater stimuli of

the same character. Glomangiomas thus represent functionally as well as morphologically organoid overgrowths.

Glomangiomas do not become malignant. Local excision gives complete and permanent relief from symptoms.

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PROBABLE GLOMANGIOMAS

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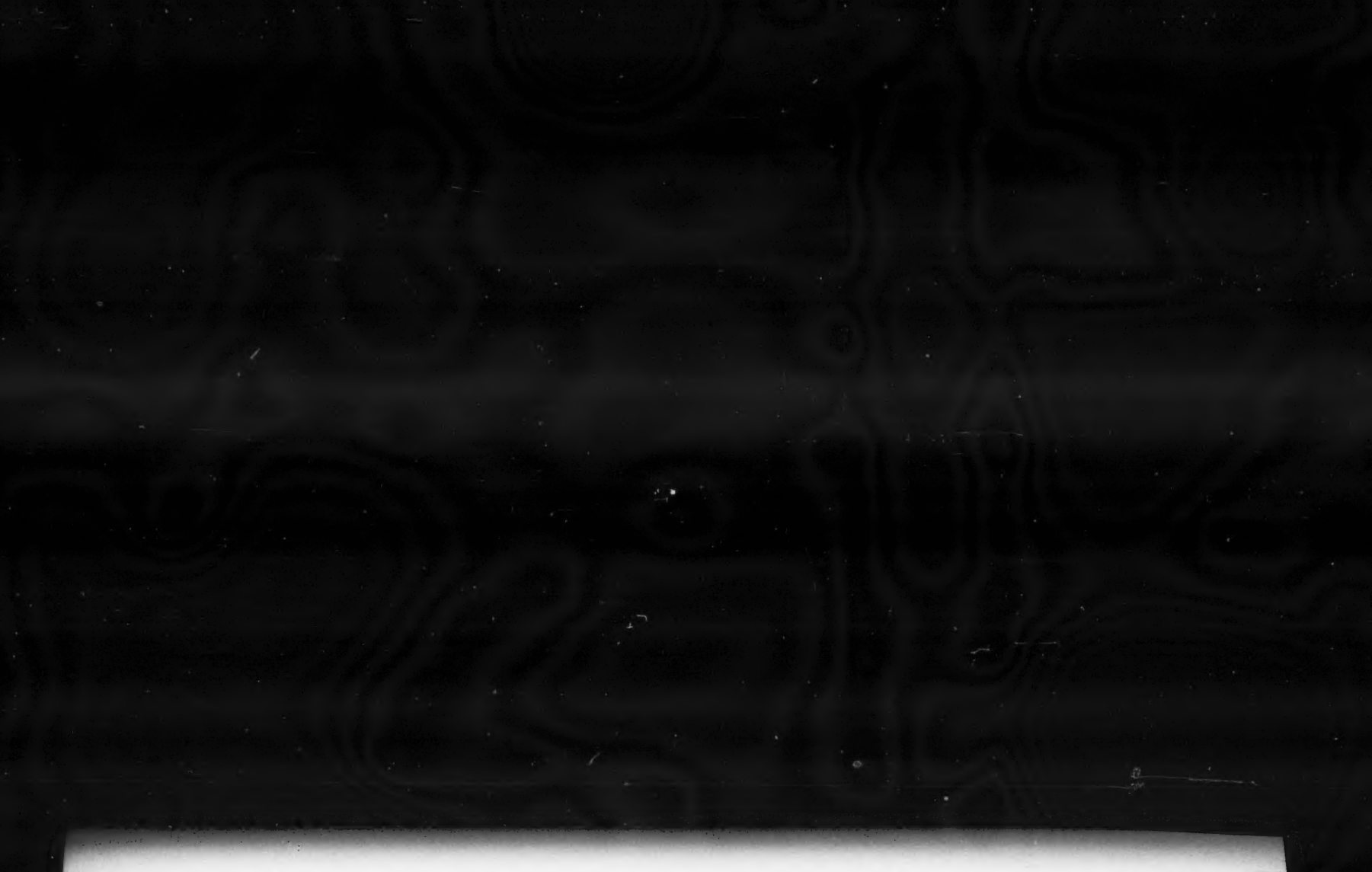
DESCRIPTION OF PLATES

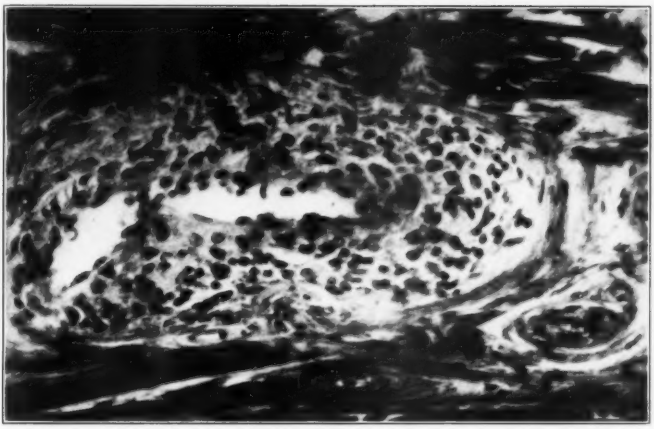
PLATE 121

FIG. 1. The normal cutaneous glomus. The specimen was obtained from the pad of the great toe of a man 36 years of age. The Sucquet-Hoyer canal has been cut longitudinally and is surrounded by a thick layer of glomus ("epithelioid") cells. At the top, to the left of the Sucquet-Hoyer canal, there is a periglomic nerve trunk. Dense connective tissue fibers surround the glomus. Mallory's phosphotungstic acid hematoxylin stain. × 215.

FIG. 2. Drawing of a subungual glomangioma (Case 7). There is no elevation or erosion of the nail. The tumor, while sharply demarcated from the surrounding tissues, is somewhat irregular in outline.

FIG. 3. The general appearance of a glomangioma at low magnification (Case 6). There are many irregular vascular lumens surrounded by glomus cells. Adjacent vessels are separated from one another by homogeneous material. The margin of the tumor is very sharply defined. Mallory's phosphotungstic acid hematoxylin stain. × 160.





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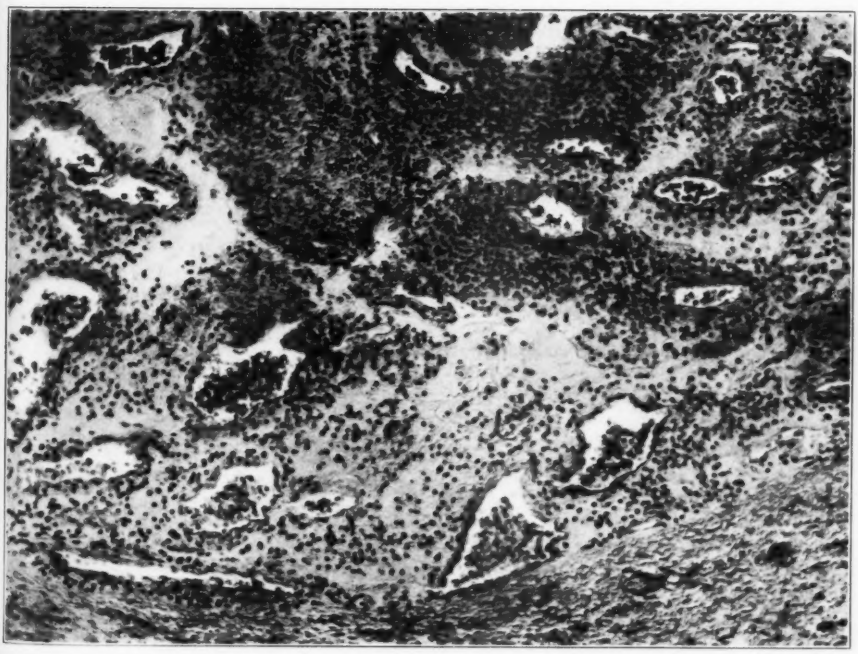

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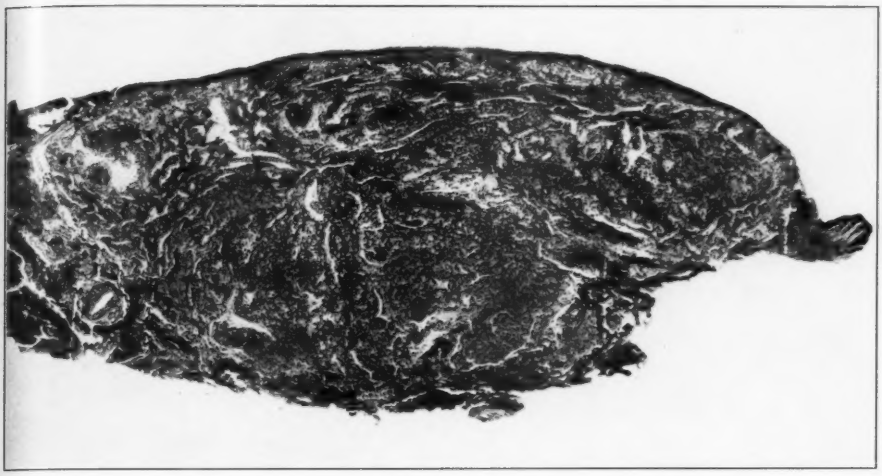
PLATE 122

FIG. 4. Section through an entire subungual glomangioma (Case 7). The uniform contour of the overlying nail bed is seen at the top. The sharp demarcation of the tumor from the subcutaneous tissues is also shown. Mallory's phosphotungstic acid hematoxylin stain. × 12 (slightly reduced).

FIG. 5. The vessels of a glomangioma (Case 5). The endothelium is surrounded by glomus cells. Some of these are separated by the homogeneous material and lie embedded in it. Hematoxylin and eosin stain. × 200.



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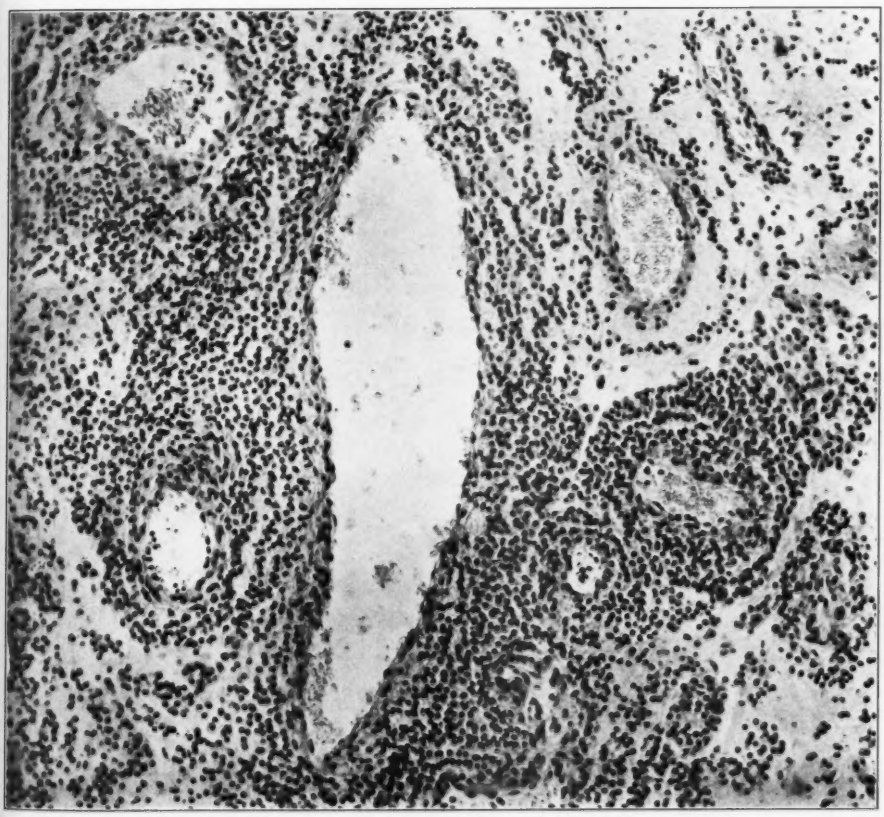

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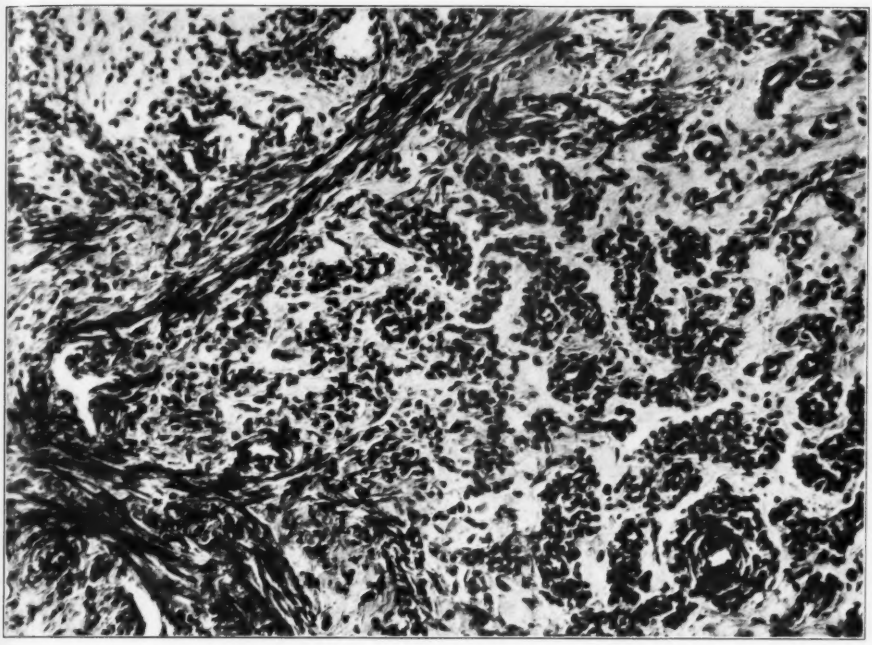
PLATE 123

FIG. 6. Large nerve trunks within a glomangioma (Case 5). Mallory's phosphotungstic acid hematoxylin stain. × 200.

FIG. 7. The entrance of a nerve trunk to a glomangioma (Case 7). A large periglomic nerve trunk bends abruptly and becomes incorporated in the tumor. Hematoxylin and eosin stain. × 175.



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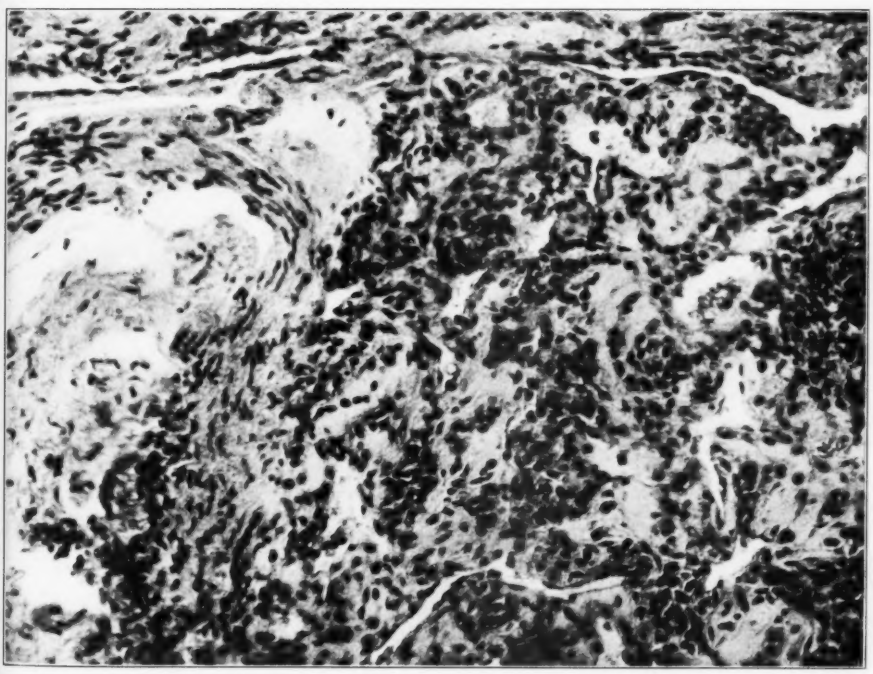

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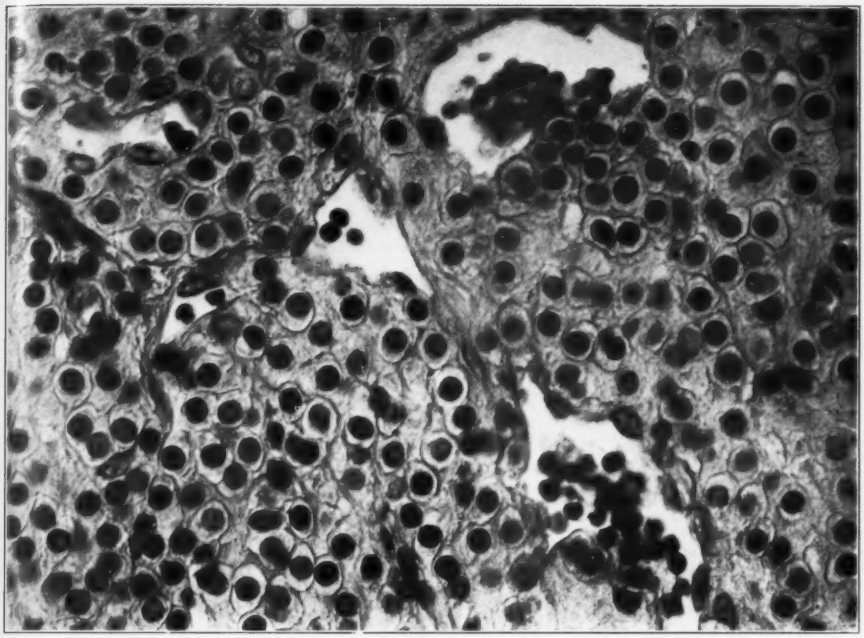
PLATE 124

FIG. 8. A mass of glomus cells with occasional vascular lumens (Case 6). The nuclei show uniformly arranged chromatin. The cytoplasm is homogeneous and surrounded by a prominent limiting membrane. Mallory's phosphotungstic acid hematoxylin stain. × 720.

FIG. 9. Camera lucida drawing of a nerve trunk and glomus cells (Case 7). A large nerve trunk with Schwannian sheath is shown at the top of the illustration. Its fibers extend among the glomus cells. A few glomus cells show nerve endings. Several contain cytoplasmic granules stained by the silver. The vascular lumen is lined by a single layer of flattened endothelial cells. Cajal's reduced silver method. × 1200.



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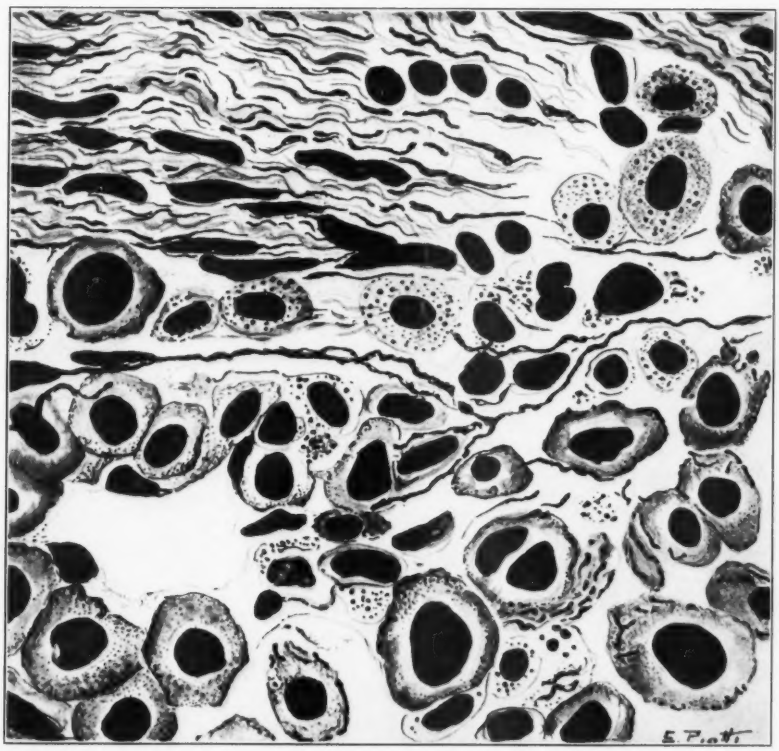

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CORNEAL REACTIONS OF NORMAL AND OF TUBERCULOUS GUINEA PIGS TO TUBERCULO-PROTEIN AND TUBERCULO-PHOSPHATIDE *

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Tissue reactions to the more purified components of the tubercle bacillus indicate that the protein fraction is responsible in a large measure for the acute exudative reaction, including much of the cellular response to the organism, and that the lipoids, particularly the phosphatide, stimulate the more chronic response and particularly the development of epithelioid cells and the lesions characteristic of tuberculosis.

Following intraperitoneal injections of adequate amounts of tuberculo-protein in normal rabbits, Doan, Sabin and Forkner [1] and Miller [2] observed an outpouring of leukocytes, clasmatocytes, plasma cells and lymphocytes in the omentum. A similar cellular response was found in the meninges of normal and of tuberculous rabbits by Bickford.[3] In some of the omentums studied by Miller plasma cells predominated. In general, they were noted by all of the above investigators as a prominent feature of the reactions. Epithelioid cells were never present in significant numbers. After intratracheal injections of tuberculo-protein Larson and Long [4] observed an exudation of polymorphonuclear and mononuclear leukocytes into the pulmonary alveoli of both normal and tuberculous guinea pigs, the more marked reaction occurring in the latter group. Seibert [5] noted a slight leukocytic response in the skin of normal guinea pigs following an injection of purified tuberculo-protein. In tuberculous animals and in those sensitized by tuberculo-protein the cellular reaction was marked even to very small amounts of the material; it consisted of many polymorphonuclear neutrophiles, large numbers of eosinophiles and mononuclears. Another striking change, as Long [6] had pointed out previously, was the marked swelling and subsequent degeneration of the connective tissue fibers. In the testes of tuberculous and tuberculo-protein sensitized guinea pigs Seibert found an interstitial edema and infiltration by numerous mononuclear exudate

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cells in response to the protein. In many instances there were polymorphonuclears and eosinophiles also. Some testes showed marked degeneration of the germinal cells. Similar changes had been noted by Long [7] after injecting Old Tuberculin into testes of tuberculous guinea pigs. In the later stage of the reaction these organs became atrophic and were infiltrated with large mononuclears. Old Tuberculin was without effect on testes of normal animals. The protein used by Seibert caused a slight leukocytic infiltration in the testes of some normal animals and none in others.

These results have shown that though relatively inert in normal tissues in small amounts, tuberculo-protein in sufficient quantity is capable of stimulating a cellular response somewhat similar to that to the same material in tuberculous animals, except in degree. In the allergic tissues is the added factor of tissue degeneration in many cases.

The biological testing of the various lipoids isolated from the tubercle bacillus has shown that the phosphatide fraction stimulates the most specific cellular reaction. Sabin [8] has given a comprehensive review of the literature concerning the activity of the tubercle bacillus lipoids in animal tissues and has summarized the extensive experiments performed by herself and her co-workers with these substances. It was found that repeated injections of the phosphatide into the peritoneal cavities of normal rabbits resulted in the production of masses of epithelioid cells, some of which were arranged to form typical tubercles. Giant cells and caseation were also observed. Injected into the subarachnoid space of normal and of tuberculous rabbits, phosphatide from bovine tubercle bacilli caused a slow production of epithelioid cells.[3] On the other hand, Boissevain [9] found evidence that tuberculo-phosphatide, if highly purified, did not bring about development of epithelioid cells or tuberculous-like tissue. Such were present when a water-insoluble protein of the tubercle bacillus was used, however. Smithburn and Sabin [10] found no tuberculous-like tissue after injecting water-insoluble protein derived from tubercle bacilli. Intracutaneously, a rather persistent nodule without any likeness to a tuberculin reaction was produced by tuberculo-phosphatide at the site of injection in tuberculous animals.[10]

Since no parallel studies had been made of the action of small amounts of tuberculo-protein and tuberculo-phosphatide in normal and allergic animals, these were undertaken. Because of its access

for continuous observation grossly, and since its avascular structure offered a favorable site for tracing inflammatory cells, the cornea was chosen as the tissue in which to study the reactions to the above materials. Guinea pigs were used. The protein and phosphatide employed were prepared from a human strain of the tubercle bacillus.

Materials

The protein was the purified TPT prepared by Dr. Florence B. Seibert from tubercle bacilli of the H37 strain by the ultrafiltration and trichloracetic acid method.[11] The phosphatide, A3-a, was also prepared by Dr. Seibert according to the method of Anderson [12] from an alcohol and ether extract of strain 119 tubercle bacilli, which was in fact a strain of H37 from another laboratory. Additional filtrations through Seitz filters were employed for removing remaining bacillary débris. There were no acid-fast particles in the final product, which easily made a permanent fine emulsion in distilled water and in normal saline solution. It contained 0.229 per cent of nitrogen of undetermined nature.

Method

A group of male guinea pigs that had been infected for 6 to 8 weeks with H37 tubercle bacilli, and which gave strong tuberculin reactions, received intracorneal injections of approximately 0.01 mg. of tuberculo-protein TPT in 0.005 cc. of normal saline. A group of normal guinea pigs was similarly injected. Another group of tuberculous and one of normal guinea pigs received approximately 0.1 mg. of the tuberculo-phosphatide A3-a emulsified in 0.005 cc. of normal saline in the cornea. One animal from each group was killed by illuminating gas at 3, 7, 15 and 30 days after injection. At later dates the experiment was repeated twice, with constant results. In one repetition animals were sacrificed at 30 hours and 11 days in addition to the above periods. The injected eyes were removed, fixed in formol-Zenker's solution and prepared for sectioning by the celloidin method. Hematoxylin and eosin stains were used routinely; hematoxylin-eosin azure and a modified Weigert fibrin stain [13] were used in certain cases.

In the subsequent descriptions the term mononuclear cell will include the various types of uninuclear cells of inflammation except

epithelioid and plasma cells. This is used because of the difficulty in accurately identifying many cells which were of intermediate or transitional types. The cells referred to as epithelioid are mononuclears with a large amount of pale, finely granular cytoplasm and a large vesicular nucleus, their shape being round to elongate, depending apparently on the degree of pressure by adjacent structures. These cells are like those seen in typical tuberculous lesions. The term fibrinoid is used to describe fibrin-like material in the anterior chamber of certain eyes which did not stain like fibrin by special methods for demonstrating this substance.

Reactions to Tuberculo-Protein

Normal Animals: Grossly at 30 hours there was a slight central cloudiness in the corneas of normal guinea pigs. This diminished rapidly and disappeared by or shortly after the third day.

Microscopically the reaction at 30 hours consisted of numerous polymorphonuclear neutrophiles between collagen bundles in a small central area of the cornea, while a few such cells were to be seen in the limbus and migrating toward the center. At 3 days small numbers of cells, a few of them mononuclears, remained, but at subsequent periods the tissue was essentially normal.

Tuberculous Animals: Grossly the cornea of the tuberculous guinea pig sacrificed 30 hours after injection was diffusely grayish white and semitranslucent. At 3 days new capillaries could be seen at the sclerocorneal junction, and the grayish white appearance was more intense, especially 1 to 2 mm. from the corneal margin. By 7 days many vessels were approaching the center, which was now white and almost opaque. Between the 9th and 15th days ulcerations with vascularized margins developed in the opaque area in seven of eight animals. The reaction in the periphery was diminishing. At 30 days all that remained was a poorly demarcated, barely visible, gray, central area traversed by a few small blood vessels.

Microscopically at 30 hours the epithelium was missing from the center of the cornea of the tuberculous guinea pig. This cornea was thickened three to four times normal because of a marked swelling of the collagen bundles and some interstitial edema; the latter was more pronounced in the limbus, where large numbers of polymorphonuclear neutrophiles were present in the interstitial spaces. Among them, but more numerous about the vessels than elsewhere, were

many mononuclears of all types from small lymphocytes and other less differentiated small cells to large, round or irregularly shaped cells with large, oval to bilobed nuclei, some containing relatively small amounts of chromatin, others coarse, dark chromatin strands. A few well defined spaces adjacent to blood vessels, perhaps lymphatics since they contained no erythrocytes, were packed with these cells and a few polymorphonuclears. Fibrous connective tissue cells and endothelial cells were swollen. Large numbers of polymorphonuclears had migrated into the cornea proper for a short distance, but few had reached the center, where swelling of the collagenous tissue was the only striking feature. In the anterior chamber, enmeshed in a delicate fibrinoid network, were many polymorphonuclears and an occasional mononuclear. Numerous cells of similar types were in the iris and its angular space.

At 3 days (Fig. 1) masses of polymorphonuclears lay in the interstitial spaces well out in the cornea but were still sparse in the center. They had decreased markedly in the limbus, mononuclears being predominant and increased greatly in number, particularly about the vessels. In some instances they filled well defined perivascular spaces. There was a definite proliferation of new capillaries, in the walls of which endothelial cells in mitosis were found frequently. The lumens of these capillaries contained polymorphonuclears and mononuclears considerably in excess of the normal number, the ratio of the two types being approximately one to one. Examples of migration of these cells through vessel walls were present. The anterior chamber contained many more leukocytes of all types and a more coarse fibrinoid network than previously. There was little change in the iris and the angular spaces. Numerous leukocytes were clustered about the ciliary body.

At 7 days many newly formed capillaries were approaching the center. Throughout the recently vascularized areas mononuclears were numerous and definitely outnumbered polymorphonuclears, while in the non-vascularized center they were still scarce. They predominated over polymorphonuclears in vessels as well, and migration of both types of cells through endothelium was taking place. In the corneal center were large numbers of polymorphonuclears, most of which were degenerating, as indicated by swollen granular cytoplasm and pyknotic nuclei. Some had been phagocytosed by the few large mononuclears present and by other polymorphonuclears.

In one cornea cells of the latter type were confined chiefly to a well localized area, while in all others they were spread diffusely through the tissue. In the limbus, inflammatory cells had diminished and throughout the cornea swelling of collagenous tissue was receding. The inflammatory process was subsiding in the anterior chamber also. Plasma cells in moderate numbers appeared in the iris of one eye.

In the one cornea studied at 11 days the chief change was an extensive ulceration and sloughing of necrotic collagenous tissue and degenerating inflammatory cells in the center. In adjacent areas the cellular constituents were mostly mononuclear, some of which were large with pale cytoplasm and large vesicular nuclei; apparently these were approaching an epithelioid-like state. There was no evidence at this or at earlier periods of local mononuclear cell formation. The few mitotic figures present were of endothelial or of fibroblastic origin as far as could be determined. In the limbus were relatively few cells. Approximately one-third were plasma cells, the remainder mononuclears.

At 15 days typical epithelioid cells and large numbers of more undifferentiated large and small mononuclears, many of them small lymphocytes, were spread diffusely through the corneal center. Whether large mononuclears and epithelioid cells were transformations of the smaller cells could not be positively determined; yet there were many intermediate forms which may well have represented successive stages of development from small mononuclears to the above types. The collagenous tissue in the center was stained lightly and had lost its laminated appearance in places, thus indicating early degeneration. The limbus was little altered from the previous period.

At 30 days the corneas were in various stages of change and recovery. In one the collagenous tissue formed a homogeneous matrix in the center. Here were suspended numerous epithelioid cells, large and small mononuclears, an occasional plasma cell and fibroblasts (Fig. 3). Phagocytosed cell débris was present in some epithelioid cells and in large mononuclears. In adjacent less degenerated areas small mononuclears were predominant. Again, transitional forms toward the epithelioid type were seen frequently. The blood vessels in the center contained fairly numerous, large and small mononuclears and a few polymorphonuclears. As at all previous periods,

some were migrating through vessel walls. Other corneas of this period showed distorted and thinned collagen bundles indicating previous injury and subsequent atrophy. The inflammatory cells had practically disappeared from these.

Reactions to Tuberculo-Phosphatide

Normal Animals: Grossly in corneas of normal guinea pigs tuberculo-phosphatide brought about a mild, but persistent, reaction characterized by a gray cloudiness which was confined to the area infiltrated with the phosphatide emulsion. The reaction reached its height at 3 days, then diminished slowly, but was still visible at 30 days.

Microscopically at 30 hours the center of the cornea was infiltrated with a moderate number of polymorphonuclear neutrophiles, while in the limbus and migrating from it toward the center were fewer polymorphonuclears and an occasional mononuclear. Most cells of the latter type were about vessels in the limbus. There was no interstitial edema or swelling of collagen bundles. At 3 days a few mononuclears were to be seen in the center, where, however, the reaction still consisted of polymorphonuclears predominantly. At 7 days many of the latter cells were degenerating, and some had been phagocytosed by mononuclears of both large and small types, which had increased in number over the previous period. A very few were migrating from the limbus. In one cornea, in which the reaction was especially marked, perivascular spaces were filled with mononuclears. Some were also in vessel lumens, and a few were passing through the endothelium. By 15 days mononuclears predominated in the center. There were transitional forms between these and epithelioid cells, a few of which were present. At 30 days the latter type of cell was predominant and lay between collagen bundles in the center. They were present in varying number in every cornea receiving the phosphatide, in some cases occupying an area of considerable size. There were no typical tubercles.

Tuberculous Animals: In corneas of tuberculous animals the tuberculo-phosphatide reaction was intense and simulated that to tuberculo-protein in many ways. The marked difference in gross and microscopically was that the phosphatide reaction was localized rather than diffuse. In both normal and tuberculous animals it was more persistent also.

In gross at 30 hours there was a marked, circumscribed, grayish white opacity in the center of the cornea, while the peripheral portions were less involved and blue-gray. The height of the reaction was reached between the 3rd and 7th days. The central opaque area assumed a dense yellowish white color and was even more sharply circumscribed than before, appearing as a spherical nodule set deeply in the corneal tissue. Numerous capillaries that had appeared at the corneal margin by the 3rd day had almost reached the center by the 7th. The peripheral reaction was subsiding at the latter period. Between the 10th and 15th days two of ten corneas developed shallow central ulcers, which persisted but a short time. The dense central inflamed area was small and the vessels less prominent. At 30 days there was only a slight, but localized, central haziness much like that in the corneas of the normal guinea pigs sacrificed at the same period, but more marked than any corresponding corneas receiving the protein.

Microscopically at 30 hours the striking features of the phosphatide reaction in tuberculous guinea pigs were the marked swelling of collagen bundles and the large number of polymorphonuclear leukocytes that filled the interstitial spaces throughout the center of the cornea. The swelling referred to was identical with that described in the tuberculo-protein reaction. The cell types were the same as well. In the limbus, mononuclears and polymorphonuclears were decidedly less numerous than in response to the protein. The same was true of the iris and its angular spaces, and there was almost no involvement of the anterior chamber.

At 3 days (Fig. 2) capillaries were proliferating extensively in the limbus and were pushing into the cornea for short distances. They contained increased numbers of mononuclears and polymorphonuclears in about equal proportions; a few of each type were passing through capillary walls. In the interstitial tissue of the limbus mononuclears showed a slight increase over the earlier period, especially about the blood vessels. In the center was a large, well localized area consisting of polymorphonuclears packed between distorted collagen fibers. Many of the cells showed signs of degeneration. The anterior chamber, iris and angular space were practically devoid of leukocytes, another indication of the definite localization of the reaction to the phosphatide in contrast with that to tuberculo-protein in tuberculous animals.

By 7 days new capillaries had reached the margin of the localized central cell mass, which now contained many mononuclears of all types. Phagocytosis of polymorphonuclears was much in evidence. In the adjacent vascularized tissue mononuclears were predominant; the same was true in the vessels. Between center and limbus as well as in the limbus itself were relatively few inflammatory cells.

In the cornea studied at 11 days the central zone was undergoing necrosis and ulceration. The adjacent vascularized tissue contained large numbers of mononuclears and, as described in the case of the corresponding animal receiving tuberculo-protein, many were approaching an epithelioid-like state. A few mature epithelioid cells were already present.

At 15 days the center was well vascularized, and epithelioid cells were numerous, if not predominant. There were still many mononuclears, especially in lateral portions of the cellular area. Some similar cells were migrating through vessel walls. The swelling of collagen bundles had practically subsided, and the limbus and other parts of the eye were essentially normal except for plasma cells in small numbers.

Thirty days after injection the corneas were identical with those of normal animals receiving phosphatide, except that a few receding vessels were still present in those of the tuberculous animals. There were epithelioid cells (Fig. 4) between collagen fibers in the center of each cornea, comprising a compact mass in some instances, but not arranged as a typical tubercle.

The marked tuberculin-like activity of tuberculo-phosphatide in corneas of tuberculous guinea pigs, in contrast with the mild reaction of normal guinea pigs to the same substance, suggested strongly that it contained tuberculo-protein. The difficulty, if not impossibility, of making an absolute separation of phospholipins and traces of protein is generally recognized, and Wells [14] has called attention to the probability of the presence of some protein in most or all of the so-called lipoid antigens of bacterial origin. Since foreign proteins mixed with lipoids often have greater antigenic power than when used alone,[15] it was thought that perhaps the tuberculo-phosphatide may have enhanced the activity of any protein which it might contain. It was suggested that a study of the reactions to tuberculo-protein mixed with a phosphatide other than that from tubercle bacilli be made.

Lecithin and Tuberculo-Protein

Mixtures of 0.1 mg. of emulsified Merck's egg lecithin with 0.0001 mg. and with 0.00001 mg. of tuberculo-protein TPT were injected into the corneas of normal and of tuberculous guinea pigs. The same amounts of these substances were injected separately into corneas of other normal and tuberculous guinea pigs for control. Histological studies were made at 3, 7, 15 and 30 days after injection.

In the normal control animals tuberculo-protein, lecithin, and their mixtures brought about only a minimal reaction of polymorphonuclears and an occasional mononuclear without definite localization. Inflammatory cells were relatively most numerous at 3 days, the corneas being practically normal at subsequent periods. In tuberculous guinea pigs lecithin gave the same result as in normal animals.

There was no constant qualitative or quantitative difference between reactions to the lecithin-protein mixtures and to the equal amounts of tuberculo-protein alone in tuberculous animals. Intensity of the reactions varied with sensitivity of the animals. Some that were cachectic did not react at all; others that were highly sensitive responded almost as intensely and with the same types of cells, swelling of collagen bundles and vascularization of the cornea as did those receiving 0.01 mg. of tuberculo-protein. In most instances the reaction was diffuse, although in a few it was somewhat localized. The same was true of the lecithin-tuberculo-protein mixtures, a few animals injected with them not reacting at all and others strongly, but no more than to the small amounts of tuberculo-protein injected alone. Some reactions were diffuse with many leukocytes in the anterior chamber and limbus; others were confined to the cornea proper.

At 15 days some epithelioid cells and numerous mononuclears were seen in the center of the corneas of those animals responding most markedly, both to lecithin-protein mixtures and to 0.0001 mg. and to 0.00001 mg. of protein alone. The above types of cells were present in appreciable numbers only after vascularization of corneal centers had taken place. Mononuclears in the vessels were more numerous than normally, and some were migrating through the endothelium. As brought out several times previously, epithelioid cells seemed to be the result of transition from mononuclears after

the latter cells had reached the reaction site, chiefly through blood vessels.

In some corneas of the various groups of animals studied at 30 hours and 3 days after injection, a few small groups of bacteria were seen in or near the path of the needle. These organisms appeared morphologically like a micrococcus commonly found in air. They were without any significant effect on the reactions, since in those corneas in which bacteria were present the reactions were the same as those in other corneas of the same groups and periods in which none were found. None were seen later than 3 days after injection, indicating that, if present at first, they had been destroyed in every case after this period, and showing that they did not alter the reactions to the materials used for study.

Discussion

In the above experiments swelling of collagen bundles in corneas of tuberculous guinea pigs receiving tuberculo-protein, also in those receiving tuberculo-phosphatide, was like that described by Seibert [5] in the cutaneous reaction of animals sensitive to the protein. The subsequent loss of structure and final partial atrophy of the collagen bundles in the protein reaction demonstrated the marked toxicity of tuberculo-protein for sensitized connective tissue. Cellular response to this material was, except for the presence of epithelioid cells, what one would expect in the reaction of any animal to an antigen with which it had been rendered allergic, namely, polymorphonuclear leukocytes at first, then large numbers of mononuclears. The appearance of epithelioid cells in appreciable numbers in later stages of the reaction was of significance, since the water-soluble protein fraction of tubercle bacilli had not been considered a stimulus for the development of these cells. Their presence in the reaction suggested that some of the epithelioid cells in lesions of tuberculosis may be due directly or indirectly to the action of tuberculo-protein on allergic tissues. Since there was no transformation to the epithelioid state of any of the few mononuclear cells responding to tuberculo-protein in corneas of normal guinea pigs, it would seem that the protein did not act on the mononuclear cells directly, but rather that some factor secondary to the degeneration of collagenous tissue or to degeneration of inflammatory cells brought about the change of

mononuclears to epithelioid cells. The real mechanism of the process is not understood.

The presence of epithelioid cells in later stages of reactions to tuberculo-phosphatide in corneas of both normal and tuberculous guinea pigs gave, without exception, further confirmation of the ability of this fraction of the tubercle bacillus to bring about the production of epithelioid cells, as Sabin and her associates have shown. Another important feature of the reactions was the early localization of responding cells in the center of the cornea, as opposed to the diffuse spreading of cells in tuberculo-protein reactions. The most logical explanation was that the phosphatide, as an emulsion, could not diffuse far from the site of injection because the globules of material could not pass through or between the collagen bundles; hence the central localization of reacting cells. The protein, as a solution, diffused from the cornea into the limbus, iris and anterior chamber, thus calling forth cells into all of these areas.

It seemed correct to assume the presence of tuberculo-protein in the phosphatide in view of its marked tuberculin-like activity in tuberculous animals, also because of its nitrogen content which, though small, might well have been due in part to protein. If this were true, then the localization of the allergic reaction to the phosphatide would indicate that the two substances were closely associated physically or chemically and that, as a result, most of the reaction due to the protein was confined to the area which the phosphatide penetrated at the time of injection. The failure of simple mixtures of lecithin and tuberculo-protein to give a localization of inflammatory cells also pointed to a close association of tuberculo-phosphatide with protein. Whether or not the phosphatide amplified the assumed protein activity by virtue of close association with it has not been determined. Because of the 0.229 per cent of nitrogen in the phosphatide, it is possible that 0.1 mg. injections of the latter contained as much as 0.0015 mg. of protein. This amount might well account for the difference in intensity between tuberculo-phosphatide reactions in normal guinea pigs and tuberculous ones.

In previous experiments [16, 17] it was shown that most of the mononuclears taking part in reactions to living tubercle bacilli injected into the corneas of guinea pigs and rabbits came into the cornea only after vascularization of it had occurred, and that these cells apparently migrated from the blood vessels. Some of them later differen-

tiated into epithelioid cells. These same observations have been made above in describing the reactions to the tuberculo-protein and phosphatide. It seemed that where there was but a slight irritation, as in the corneas of normal guinea pigs, sufficient numbers of mononuclears were able to migrate from the limbus without the aid of vascularization. Normally the limbus contains small numbers of lymphocytes and other mononuclears to supply some cells for mild injuries. In the presence of severe injuries, however, vascularization was apparently necessary to supply the needed mononuclear cells. Polymorphonuclears were able to migrate in large numbers from the limbus early in the reactions; mononuclears were not. In no case was there evidence of local proliferation of inflammatory cells, there being no mitotic figures except in endothelium and occasionally in cells which seemed surely to be fibroblasts. Neither was amitotic cell division observed. More positive indication of the vascular source of the mononuclears was their increased number in vessel lumens and the observation of some of them migrating through vessel walls. The fact that frequently they were most numerous about vessels was also in favor of their vascular entrance to the site of inflammation.

That epithelioid cells developed from mononuclears after these had reached the area of inflammation seemed to be demonstrated by the absence of epithelioid cells in the lumens of vessels; and the presence in the inflamed tissue of cells apparently in transition from mononuclear to epithelioid form further supported their mononuclear cell origin.

Summary and Conclusions

A study of the tissue reactions to purified tuberculo-protein and tuberculo-phosphatide in the corneas of normal and tuberculous guinea pigs was made over a period of 1 month. It was found that tuberculo-protein had a markedly toxic action on the connective tissue of corneas of tuberculous animals and led to inflammation and partial degeneration (tuberculin reaction). Furthermore, it seemed responsible, probably indirectly, for the production of epithelioid cells in the later stages of the allergic reactions. In the amounts used, the protein was practically inert in normal guinea pigs.

Tuberculo-phosphatide also caused an acute tuberculin-like reaction in tuberculous guinea pigs. Inasmuch as the preparation con-

tained a small amount of nitrogen, believed to be an impurity, and not part of the molecule, it was concluded that the reaction noted was probably a reaction to tuberculo-protein. The fact that the unknown substance was closely bound chemically seemed to explain the fact that the acute reaction to the tuberculo-protein alone was diffuse, while that to the tuberculo-phosphatide was localized. In both tuberculous and normal animals epithelioid cells were present at the later periods and persisted longer than did those in most of the tuberculo-protein reactions.

The findings confirmed previous work indicating that in tuberculosis of the cornea most mononuclears taking part in the reactions at the site of injection came from the blood stream, and that epithelioid cells arose from these mononuclears by a process of transition at the site of inflammation.

NOTE: The writer is indebted to Dr. Esmond R. Long for direction and other aid in the experiments presented and to Dr. Florence B. Seibert for supplying the tuberculo-protein and tuberculo-phosphatide.

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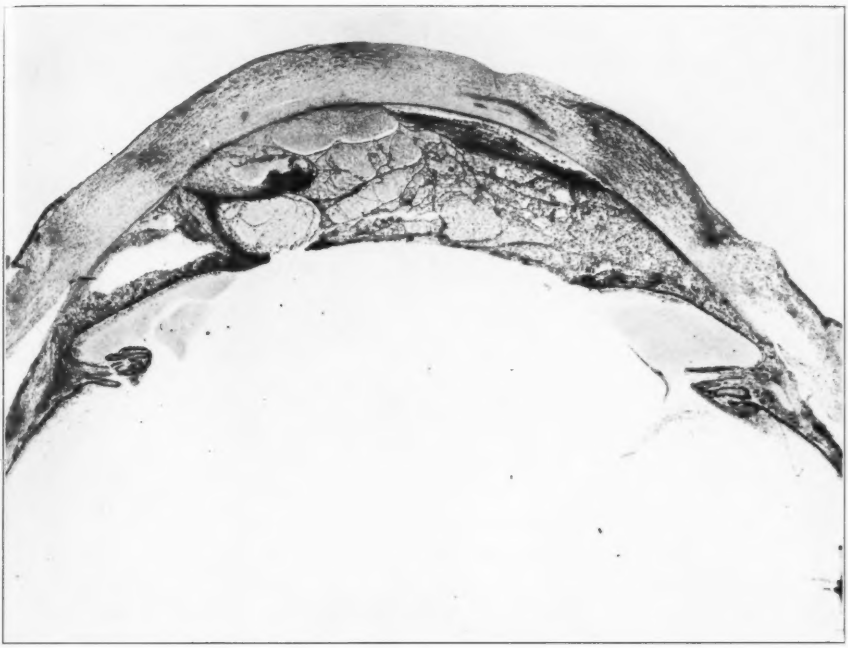
DESCRIPTION OF PLATES

PLATE 125

FIG. 1. Cornea of tuberculous guinea pig 3 days after central injection of tuberculo-protein. Note marked density in and near limbus due to cellular infiltration, and relatively slight infiltration in center. Note also heavy exudate in anterior chamber. × 13.5.

FIG. 2. Cornea of tuberculous guinea pig 3 days after central injection of tuberculo-phosphatide. Note dense cell "mass" in center and absence of exudate in anterior chamber. × 13.5.



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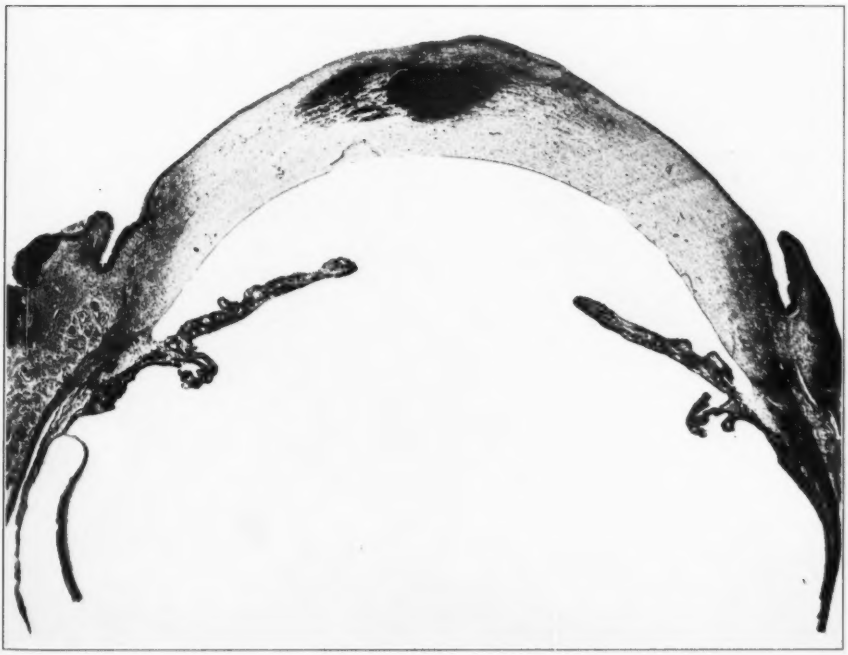

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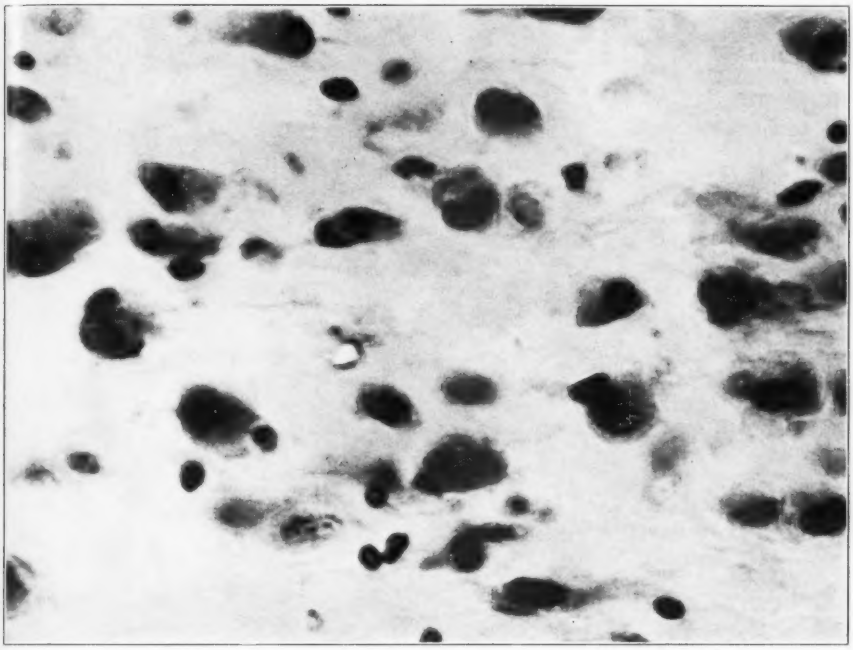
PLATE 126

FIG. 3. Center of cornea of tuberculous guinea pig 30 days after central injection of tuberculo-protein. Note epithelioid and other less differentiated mononuclear cells in homogeneous matrix of degenerated collagenous tissue. × 910.

FIG. 4. Center of cornea of tuberculous guinea pig 30 days after central injection of tuberculo-phosphatide. Note large number of epithelioid cells and interspersed less differentiated mononuclears, some possibly in transition toward epithelioid type. × 910.



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THE SIGNIFICANCE OF THE CELLULAR VARIATIONS OCCURRING IN NORMAL SYNOVIAL FLUID *

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In order to establish the cytological characteristics of normal human synovial fluid studies have been made on human synovial fluid obtained immediately after death.[1] Such studies reveal considerable variation in the total number of nucleated cells as well as percentage differences of any one type of cell. Therefore, it is apparent that one cannot properly interpret these data until one has determined what cytological variations can occur and still be within the limits of normal. Because cellular variations of a lesser degree had been observed previously in bovine synovial fluid [2] further studies of bovine fluid were undertaken in order to determine if possible not only what magnitude of cellular variation can exist without being considered abnormal, but also what factors are responsible for the observed cellular variations. In order to determine further the influence of certain factors on synovial fluid cytology some of the observations were extended to dogs because greater variations of the suspected factor were possible. Certain observed discrepancies between the present and previous bovine fluid studies [2] were noted and have been recorded.

METHODS

Cell counts were recorded on 37 specimens of synovial fluid from young beef cattle. Of these, 25 specimens were obtained from the astragalotibial joint and 12 specimens were taken from the carpometacarpal joint. The method employed in the present study differed only slightly from that previously described.[2] It was found

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that better differential stains were obtained if the concentration of vital dyes was reduced. Therefore, 12 drops of a saturated solution of neutral red in absolute alcohol and 4 drops of a saturated solution of Janus green B in absolute alcohol were added to 10 cc. of absolute alcohol. Clean glass slides were flooded with this diluted stain, drained and allowed to dry quickly.

In order to compare the phagocytic activity of each cell observed with its reaction to the vital dyes differential cell counts were made on two portions of each fluid, one of which contained a few drops of a graphite suspension. The same concentration of vital dyes was used in each type of preparation. The cell counts were then made simultaneously by two observers.

Results

The results of the present series of cell counts are presented in Tables I and II. A comparison of the average percentage of the various types of cells listed in these tables with those previously published [2] reveals that there is a much higher percentage of non-phagocytic cells than we formerly reported. This group of non-phagocytic cells is comprised chiefly of cells having the characteristics of lymphocytes.[3, 4] It will be noted that 20 to 23 per cent of all nucleated cells in the carpometacarpal joint fluid and 40 per cent in the astragalotibial joint fluid are lymphocytes, whereas we formerly reported that 90 to 95 per cent of all nucleated cells in synovial fluid from these two joints were phagocytic. The averages of the present tabulation indicate that only 74 to 78 per cent * of the nucleated cells in the synovial fluid from the carpometacarpal joint and 57 per cent of the nucleated cells in the astragalotibial joint fluid are phagocytic cells. These tables also show a wide range of variation in the numbers of phagocytic and non-phagocytic cells in different specimens of synovial fluid from the same source. No significant difference was noted between the average number of polymorphonuclear leukocytes and synovial cells in the two studies. A somewhat higher average of nucleated cells per cubic millimeter of astragalotibial joint fluid was obtained in the present series of counts. The former series of 63 specimens showed an average of 112 nucleated cells per cubic millimeter, whereas the average number of nucleated cells per cubic millimeter

* 74 per cent was the average for the fluids without graphite and 78 per cent represents the average for the same fluids when graphite was present.

of fluid in the present series is found to be 181. This difference is due chiefly to the fact that 4 specimens of fluid (Nos. 5, 13, 16 and 17) in the present series were unusually rich in cells.

Discussion

Re-examination of the cells in the synovial fluid of cattle by means of supravital staining has demonstrated an error in our previously reported observations [2] in which 90 to 95 per cent of the nucleated cells were classified as phagocytes. The existing discrepancy resulted from a failure to distinguish between lymphocytes and small monocytes and from the belief that certain lymphocytes were shrunken degenerating cells. This source of error has been largely removed by reducing the concentration of vital dyes and by observing the function of each cell with regard to its ability to ingest particulate matter. In the previous study graphite was added to several synovial fluids for the purpose of making illustrations of the various types of cells contained therein. Had differential cell counts been made on these preparations we would not have made the mistake of classifying certain lymphocytes as monocytes.

The present series of differential counts was checked in each instance by preparations to which particulate matter had been added. Although the addition of a graphite suspension materially aided in the early stages of this study in the differentiation of phagocytic from non-phagocytic cells, it interfered greatly with the further classification of the phagocytic cells. This fact is clearly demonstrated by a comparison of the percentages of unclassified phagocytes counted in preparations without the addition of graphite, with the percentages of such cells obtained in counts made after such particulate matter had been added.

At the present time we know of no real value in subdividing the large mononuclear phagocytes of synovial fluid. However, an attempt has been made to classify them according to the criteria given for their differentiation [5] in order to record the relative percentages of clasmatocytes and monocytes for future comparison with counts made on normal and pathological human synovial fluids.

In counting a specimen of synovial fluid one frequently encounters dead or degenerating cells. These are distinguished by their failure to react to vital dye, by their hyaline nuclei which are sometimes stained pale green, and by their shrunken or excessively vacuolated

cytoplasm. Such cells may resemble cells of the phagocytic series or they may resemble lymphocytes. Frequently, however, no clue to their identity is evident. All such cells have been excluded from these differential counts, although the number seen in counting 11 specimens of astragalotibial joint fluid and 7 specimens of carpometacarpal joint fluid has been recorded. From 2 to 24 such cells were seen in counting 100 viable cells.

Examination of Tables I and II reveals considerable variation in the average cell percentages in carpometacarpal and astragalotibial joint fluids. Seventy-four to 78 per cent of all cells present in the carpometacarpal joint are phagocytic, whereas in the astragalotibial joint fluid only 57 per cent are phagocytic. One notes further that there is less variation in the number of phagocytic and non-phagocytic cells present in carpometacarpal joint fluid. Is there any obvious explanation for the observed cellular differences in these two joint fluids? In a previous study [6] it was noted that a degenerative type of articular cartilage defect is present in the carpometacarpal joints of all cattle over 2 years of age whereas, in so far as we could determine, no such articular cartilage lesion is present in the astragalotibial joints. Because of the presence of this cartilage defect the carpometacarpal joint fluids contain more débris and unidentified solid matter than the astragalotibial joint fluids. Such débris and particulate matter is removed from the joint chiefly by means of active phagocytic cells. Thus, it must be assumed that the increased mononuclear phagocytic cell content of carpometacarpal joint fluid represents the response necessary for the removal of the wear and tear products resulting from repeated trauma to this joint.

The variations in cell percentages observed in one astragalotibial joint fluid as compared with another were very great (Table II). The phagocytic cells varied from 36 to 92 per cent, whereas non-phagocytic cells (lymphocytes) varied from 8 to 64 per cent. Can variations of this degree be considered within the limits of normal? To answer this question it was necessary to consider all possible factors that might have been responsible for the variations noted. The following were considered and investigated:

(1) Variations resulting from failure to withdraw the fluid at the time the animal was sacrificed.

(2) Variations resulting from failure to examine the fluid immediately after its withdrawal.

TABLE I

Cytology of Synovial Fluid from Carpometacarpal Joints

Animal No.	Nucleated cells	Erythrocytes	(A) Differential cell counts before the addition of graphite										(B) Differential cell counts after the addition of graphite									
			Phagocytic cells				Non-phagocytic cells			Totals			Phagocytic cells				Non-phagocytic cells			Totals		
			Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified mononuclear phagocytes	Lymphocytes	Synovial cells	Unclassified	Phagocytic cells	Non-phagocytic cells	Dead cells seen in counting 100 nucleated cells	Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified mononuclear phagocytes	Lymphocytes	Synovial cells	Unclassified	Phagocytic cells	Non-phagocytic cells	Dead cells seen in counting 100 nucleated cells
	per cmm.	*per cmm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
1.....	555	150	0	42	10	2	44	0	2	54	46	..	0	32	2	18	46	0	2	52	48	..
2.....	305	5	0	66	8	0	24	2	0	74	26	..	0	54	18	10	18	0	0	82	18	..
3.....	205	105	0	54	8	0	38	0	0	62	38	..	0	32	8	20	38	2	0	60	40	..
4.....	170	25	4	52	6	0	38	0	0	62	38	..	0	28	8	32	22	2	8	68	32	..
5.....	225	470	0	60	14	8	14	2	2	82	18	..	0	58	8	22	10	0	2	88	12	..
6.....	210	25	2	72	10	4	12	0	0	88	12	24	2	36	14	36	12	0	0	88	12	22
7.....	120	10	0	66	8	6	16	2	2	80	20	6	0	34	8	30	24	0	4	72	28	14
8.....	120	10	2	64	8	2	20	2	2	76	24	14	0	48	10	28	12	0	2	86	14	8
9.....	100	25	0	80	2	2	14	2	0	84	16	16	0	42	10	30	14	2	2	82	18	14
10.....	275	570	4	72	6	4	8	4	2	86	14	10	2	34	6	48	6	2	2	90	10	16
11.....	125	100	2	68	6	6	12	6	0	82	18	14	0	30	10	50	6	4	0	90	10	20
12.....	150	15	0	60	0	2	36	0	2	62	38	24	0	44	2	28	26	0	0	74	26	14
Max....	555	570	4	80	14	8	44	6	2	88	46	24	2	58	18	50	46	4	8	90	48	22
Min....	100	5	0	42	0	0	8	0	0	54	12	6	0	28	2	10	6	0	0	52	10	8
Aver. ..	213.3	125.8	1.2	63	7.2	3	23	1.7	1	74.3	25.7	15.4	0.3	39.3	8.7	29.3	19.5	1	1.8	77.7	22.3	15.4

(3) Whether or not the cytology of synovial fluid is altered by variations in the blood cytology.

Postmortem increases in the total number of synovial fluid cells have been reported by Key.[7] He ascribes this increased cellular content as being due chiefly to a migration of polymorphonuclear leukocytes into the joint. The fluid cytology herein reported was done on fluids drawn 15 to 30 minutes after death because it had been established previously [2] that this lapse of time in no way influenced the total cell count or the individual cell percentages. Polymorphonuclear leukocytes were rarely observed.

In order to determine what changes in synovial fluid occur postmortem, specimens of synovial fluid from the knee joints of four normal dogs were examined at varying intervals after death. Six fluids were obtained within the first half hour, 3 at the end of 1, 2½ and 3 hours respectively, and 4 at the end of 18 hours. In four instances sufficient fluid was obtained at a later aspiration (1 at the end of 2½ hours and 3 at the end of 18 hours) to repeat the total cell and differential counts. In 3 of these later fluids a marked increase in the total number of cells occurred without any appreciable alteration in individual cell percentages. Thus we are unable to ascribe percentage variations of any one type of cell as being due to postmortem changes or intra-articular migration.

In order to determine the magnitude of variations in the differential cell counts which might result because of failure to examine the fluid immediately after its withdrawal a number of fluids were re-examined 2½ to 3 hours after the first count was made. Invariably such counts revealed a marked increase in the number of dead or degenerating cells. Considerable clumping of cells occurred when the fluids were kept in the warm box for such long periods of time. Aggregates of 5 to 50 cells were frequently observed. While the presence of such clumps did not alter the relative percentages of phagocytic and non-phagocytic cells in the specimens re-examined it was at times difficult or impossible to recognize the distinguishing characteristics of the phagocytic cells.

In the course of this study we observed in three instances cytological synovial fluid changes which suggested that the synovial fluid cytology might be a reflection of the blood cytology. For instance, in 2 pathological bovine synovial fluids an eosinophilic polymorphonuclear leukocytosis of 12 and 50 per cent respectively was noted.

TABLE II

Cytology of Synovial Fluid from Astragalotibial Joints

Animal No.	Nucleated cells	Erythrocytes	(A) Differential cell counts before the addition of graphite										(B) Differential cell counts after the addition of graphite									
			Phagocytic cells				Non-phagocytic cells			Totals			Phagocytic cells				Non-phagocytic cells			Totals		
			Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified mononuclear phagocytes	Lymphocytes	Synovial cells	Unclassified	Phagocytic cells	Non-phagocytic cells	Dead cells seen in counting 100 nucleated cells	Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified mononuclear phagocytes	Lymphocytes	Synovial cells	Unclassified	Phagocytic cells	Non-phagocytic cells	Dead cells seen in counting 100 nucleated cells
	per cmm.	*per cmm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
1	135	225	6	32	16	4	42	0	0	58	42	..	2	18	18	18	40	2	2	56	44	..
2	140	25	3	33	2	0	62	0	0	38	62	..	0	18	8	8	64	2	0	34	66	..
3	85	10	0	30	30	2	34	4	0	62	38	..	0	20	16	28	32	4	0	64	36	..
4	125	40	0	38	24	8	22	6	2	70	30	..	0	12	8	24	52	4	0	44	56	..
5	395	60	0	18	16	2	62	2	0	36	64	..	0	16	14	12	58	0	0	42	58	..
6	170	50	4	29	23	8	34	0	2	64	36	..	0	10	13	32	40	2	3	55	45	..
7	210	105	0	12	28	0	60	0	0	40	60	..	0	20	13	23	39	5	0	56	44	..
8	105	12	0	24	24	0	50	2	0	48	52	..	0	4	16	30	44	4	2	50	50	..
9	140	5	0	32	36	0	28	2	2	68	32	..	0	24	18	22	36	0	0	64	36	..
10	...	...	0	42	12	0	44	2	0	54	46	..	0	16	16	22	46	0	0	54	46	..
11	85	935	6	44	12	0	34	2	2	62	38	..	0	16	6	20	58	0	0	42	58	..
12	230	10	8	28	22	16	24	0	2	74	26	14	4	22	28	18	24	2	2	72	28	..
13	370	640	7	54	28	3	5	1	2	92	8	7	8	30	24	30	8	0	0	92	8	..
14	115	50	2	38	14	4	42	0	0	58	42	8	6	30	16	24	24	0	0	76	24	..
15	175	65	2	30	10	14	36	2	6	56	44	6	2	26	10	16	46	0	0	54	46	12
16	575	45	2	46	6	8	38	0	0	62	38	4	2	26	6	26	36	0	4	60	40	2
17	315	5	0	50	12	2	36	0	0	64	36	2	0	28	4	38	28	2	0	70	30	2
18	160	45	0	36	8	2	50	4	0	46	54	8	0	28	10	16	42	0	4	54	46	6
19	85	5	2	32	6	0	58	0	2	40	60	28	0	14	6	18	54	2	6	38	62	22
20	55	90	2	52	4	4	36	2	0	62	38	14	0	16	4	35	41	2	2	55	45	8
21	260	45	4	40	0	0	56	0	0	44	56	26	0	10	0	34	54	0	2	44	56	20
22	125	50	0	60	2	0	32	0	4	62	38	14	0	18	6	24	48	0	4	48	52	16
23	60	740	0	48	12	6	34	0	0	66	34	6	0	20	10	36	30	4	0	66	34	8
24	120	95	6	28	16	8	40	0	2	58	42	16	2	12	4	48	32	0	2	66	34	14
25	130	35	*1	34	13	6	42	1	3	54	46	2	0	34	8	26	30	2	0	68	32	2
Max. ...	575	935	8	54	36	16	62	6	6	92	64	28	8	34	28	48	64	5	6	92	66	22
Min. ...	55	5	0	12	0	0	5	0	0	36	8	2	0	4	0	8	8	0	0	34	8	2
Aver. ..	181.8	141.1	2.2	36.4	15	3.9	40.1	1.2	1.2	57.5	42.5	11.1	1	19.5	11.3	25.1	40.2	1.5	1.3	57	43	10.2

* Eosinophile.

Because only one eosinophilic polymorphonuclear leukocyte had ever been observed in over 100 bovine synovial fluid examinations it was only natural to wonder if the above findings were indicative of an eosinophilia in these two animals. In addition, examination of the synovial fluid from a patient with myelogenous leukemia revealed that many of the synovial fluid cells were of the myelocytic series. If the synovial fluid does reflect the cytology of the blood under such conditions then there would be every reason to believe that diurnal variations in the synovial fluid cytology similar to those in blood cytology might occur.

In order to prove or disprove this theory the following observations were made:

(1) Simultaneous blood and astragalotibial synovial fluid examinations were done on eight additional cattle. These results are shown in Table III. One notes that variations of the total number of circulating leukocytes (4,200 to 10,800) or variations in number of any one type of cell can occur without any reflection of such variations in the synovial fluid. For instance, the polymorphonuclear leukocytes of the blood in one animal averaged 66 per cent, yet the synovial fluid contained none. In one other animal these cells averaged 2 per cent in the synovial fluid, even though the blood contained only 10 per cent. The same absence of relationship was demonstrable in the case of lymphocytes and eosinophilic polymorphonuclear leukocytes. The blood of each animal showed an eosinophilia varying from 2 to 12 per cent, yet no eosinophilic polymorphonuclear leukocytes were demonstrable in the synovial fluid.

(2) Similar studies made on simultaneously obtained canine blood and synovial fluid gave similar results to those obtained on cattle (Table IV). Again there was no evidence of the synovial fluid cytology being influenced by the blood cytology. An eosinophilia was present in some instances and again no eosinophilic polymorphonuclear leukocytes were present in the synovial fluid.

(3) Further proof that synovial fluid cytology is not influenced by blood cytology was obtained when the same studies were repeated on normal dogs before and during the time of an experimentally produced polymorphonuclear leukocytosis. Any increase in synovial granulocytes during such a leukocytosis would be of significance because normally they are rarely present in the synovial fluid (the average being 1.7 per cent — see Table IV). As can be seen from Table V

TABLE III

A Comparison of the Cytology of Simultaneously Obtained Bovine Blood and Synovial Fluid *

Animal No.	Material	Nucleated cells	Phagocytic cells				Non-phagocytic cells			
			Neutrophilic polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified phagocytes	Lymphocytes	Synovial cells	Unclassified cells	Eosinophilic polymorphonuclear leukocytes
		per cmm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Blood	4800	48	4	..	..	46	..	0	2
	S. F.	130	0	60	4	2	26	8	0	0
2	Blood	6000	66	2	..	..	30	..	0	2
	S. F.	170	0	52	6	6	32	4	0	0
3	Blood	7100	10	6	..	..	76	..	0	8
	S. F.	115	2	74	2	8	10	2	0	0
4	Blood	4200	18	10	..	..	66	..	0	6
	S. F.	105	0	44	6	0	48	2	0	0
5	Blood	5000	28	4	..	..	60	..	0	8
	S. F.	180	2	48	8	2	40	0	0	0
6	Blood	5100	22	6	..	..	67	..	0	4
	S. F.	120	0	60	4	4	30	2	0	0
7	Blood	10800	26	4	..	..	68	..	0	2
	S. F.	220	0	28	0	0	72	0	0	0
8	Blood	6700	42	6	..	..	40	..	0	12
	S. F.	60	0	46	4	4	40	2	2	0

* The synovial fluid was obtained from the astragalotibial joints.

TABLE IV

A Comparison of Simultaneously Obtained Canine Blood and Synovial Fluid

Dog No.	Source	Totals			Phagocytic cells				Non-phagocytic cells			
		Nucleated cells	*Erythrocytes	Dead cells seen in counting 100 nucleated cells	Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified phagocytes	Lymphocytes	Synovial cells	Unclassified	Eosinophilic polymorphonuclear leukocytes
		per cmm.	*per cmm.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1.....	Blood	11600	...	..	59	12	..	..	27	..	..	2
	Right knee	327	155	3	0	72	4	0	21	3	0	0
2.....	Blood	9500	...	..	76	6	..	..	18	..	0	0
	Right knee	...	++	0	2	56	8	8	22	4	0	0
3.....	Blood	11700	...	..	55	9	..	..	36	..	0	0
	Left knee	1450	++	0	0	76	0	0	22	2	0	0
4.....	Blood	15100	...	..	90	7	..	..	3	..	0	0
	Right knee	1260	1080	0	1	90	0	0	7	2	0	0
5.....	Blood	14500	...	..	86	9	..	..	5	..	0	0
	Right knee	1080	360	0	7	66	6	5	8	8	0	0
6.....	Blood	8100	...	..	71	2	..	..	24	..	0	3
	Left knee	450	125	2	0	68	4	3	16	9	0	0
7.....	Blood	19600	...	..	75	3	..	..	13	..	0	9
	Right knee	1175	105	2	2	66	6	4	19	3	0	0
	Left knee	1360	45	3	1	62	4	6	26	1	0	0
8.....	Blood	13000	...	..	60	4	..	..	36	..	0	0
	Right knee	825	250	0	0	69	3	0	21	7	0	0
	Left knee	1115	160	0	0	58	2	0	36	4	0	0
9.....	Blood	11400	...	..	83	5	..	..	6	..	0	6
	Right knee	...	+	0	2	76	12	2	4	4	0	0
	Left knee	565	45	0	0	66	10	6	12	6	0	0
10.....	Blood	11600	...	..	58	10	..	..	25	..	0	7
	Right knee	...	+	0	0	64	12	14	2	8	0	0
	Left knee	995	400	0	0	70	20	0	4	6	0	0
Maximum...	Blood	19600	...	..	90	12	..	..	36	..	0	9
	Synovia	1450	...	3	7	90	20	14	36	9	0	0
Minimum...	Blood	8100	...	..	55	2	..	..	3	..	0	0
	Synovia	327	...	0	0	56	0	0	2	1	0	0
Average	Blood	11610	...	0	71.3	5.7	..	..	19.3	..	0	2.7
	Synovia	963.8	...	0.7	1.7	68.5	6.5	3.4	15.7	4.8	0	0

* When erythrocytes were too numerous to count the number present is represented by + signs.
All differential cell counts of blood were made from fixed smears stained with Wright's stain.

Average	Synovia	963.8	...	0.7	1.7	68.5	6.5	3.4	15.7	4.8	0	0

* When erythrocytes were too numerous to count the number present is represented by + signs.
All differential cell counts of blood were made from fixed smears stained with Wright's stain.

TABLE V

A Comparison of the Cytology of Simultaneously Obtained Canine Blood and Synovial Fluid Before and During an Experimentally Produced Leukocytosis *

Dog No.	Time cell counts were made	Source	Totals			Phagocytic cells				Non-phagocytic cells			
			Nucleated cells	Erythrocytes	Dead cells seen in counting 100 nucleated cells	Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified phagocytes	Lymphocytes	Synovial cells	Unclassified non-phagocytes	Eosinophilic polymorphonuclear leukocytes
			per cmm.	*per cmm.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	(1) Before injection of sodium nucleinate	Blood	9300	...	..	67	8	..	..	18	..	0	7
		Right knee	...	+++	0	6	81	3	2	2	6	0	0
	(2) Eight hours after injection of sodium nucleinate	Blood	19600	...	0	95	4	..	..	1	..	0	0
		Left knee	990	155	4	5	76	2	4	5	8	0	0
II	(1) Before injection of sodium nucleinate	Blood	13950	...	0	77	10	..	..	10	..	0	3
		Left knee	...	+++	0	15	66	6	3	9	1	0	0
	(2) Fourth day of leukocytosis varying from 16 to 20,000 with 84 to 92 per cent polymorphonuclear leukocytes	Blood	19400	...	0	84.3	11.3	..	..	3	..	0	1.3
		Right knee	1625	40	0.5	0.5	48	6.5	2	39.5	3	0.5	0
		Left knee	1620	++	1	0	53	0	2	36	8	1	0
III	(1) Before injection of sodium nucleinate	Blood	8400	...	0	79	4	..	..	17	..	0	0
		Left knee	810	610	0	0	69	11	0	15	5	0	0
	(2) Fourth day of leukocytosis varying from 14 to 23,000 with 86 to 88 per cent polymorphonuclear leukocytes	Blood	23200	...	0	87	4	..	..	7	..	0	2
		Right knee	980	++	0	0	68	10	8	6	8	0	0
		Left knee	1410	40	1	0	74	13	1	7	5	0	0

* The experimental leukocytosis was produced by the injection of sodium nucleinate intravenously. In Dog I a single dose of 12 gm. was given whereas, in Dogs II and III, 2 to 6 gm. were administered each day for 4 days.

no increase in synovial fluid granulocytes occurred, whether the increased number of circulating polymorphonuclear leukocytes was of short duration (8 hours in Dog I) or of many hours duration (96 hours in Dogs II and III). Again, no eosinophilic polymorphonuclear leukocytes were demonstrable in the synovial fluid. In Dog II the number of synovial fluid lymphocytes was greatly in excess of the blood lymphocytes.

From these last three experiments it would seem that one is justified in concluding that there is no evidence to support the theory that synovial fluid cytology is a reflection of the blood cytology. The findings in the 3 pathological fluids previously cited must have been due to other causes, such as parasitic invasion of the periarticular structures in the case of the cattle and subperiosteal leukemic infiltration near the joint margins in the case of the human.

The data contained in Tables II to V inclusive offer no satisfactory explanation for the wide variations noted in average cell percentages in normal bovine synovial fluid, particularly those observed in the fluid obtained from the astragalotibial joint. The presence of a regularly occurring cartilage defect in the carpometacarpal joint and in consequence more cellular débris to be removed would seem sufficient stimulus for an increase in the total number of nucleated cells and for an increased mononuclear phagocytic cellular reaction in this joint, whereas in the astragalotibial joint no such regularly occurring pathological lesion is demonstrable and therefore there is less need for an increase in this type of phagocytic cell. The variations in phagocytic and non-phagocytic cell percentages found in the astragalotibial joint fluid probably do represent the cellular variations which can take place in any so-called normal joint. Therefore, these variations probably represent cellular reactions to the average every day insults such as wear and tear, minor trauma or any irritant, any one of which all joints must be subjected to from time to time. In other words, the cytology of normal synovial fluid is dependent in part upon what intra-articular insults have occurred. For instance, the intra-articular injection of a mild irritant such as normal saline results in a marked increase in polymorphonuclear leukocytes, only to be largely replaced in a few days by mononuclear phagocytes and later by an increase in the average number of lymphocytes.[8, 9] Any joint subjected to unusual or constant use will show considerable evidence of wear and tear manifested by intra-articular degenerative

TABLE VI

Showing the Average Cellular Variations in the Synovial Fluid of Different Species

Animal	Joint	No. fluids examined	Nucleated cells	Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified phagocytes	Lymphocytes	Synovial cells	Unclassified non-phagocytes	Total phagocytes	Total non-phagocytes
			per cmm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Cow	Astragalotibial	25										
	Maximum		575	8	54	36	16	62	6	4	92	64
	Minimum		55	0	12	0	0	5	0	0	36	8
	*Average		181.8	2.2	36.4	15	3.9	40.1	1.2	1.2	57.5	42.5
Cow	Carpometacarpal	12										
	Maximum		555	4	80	14	8	44	6	2	88	46
	Minimum		100	0	42	0	0	8	0	0	54	12
	*Average		213.3	1.2	63	7.2	3	23	1.7	1	74.3	25.7
Dog	Knee	14										
	Maximum		1450	7	90	20	14	36	9	0	92	40
	Minimum		327	0	56	0	0	2	1	0	60	8
	Average		963.8	1.7	68.5	6.5	3.4	15.7	4.8	0	82.4	17.6
Rabbit	Knee	9										
	Maximum		330	15	77	26	22	6	6	4	100	12
	Minimum		140	0	48	2	8	0	0	0	88	0
	Average		242.5	2.2	65.5	12.7	13	1.5	4.2	0.7	93.5	6.5

* The average cell percentages for the astragalotibial and carpometacarpal joint fluid are taken from Tables I and II respectively and represent the differential cell counts done before adding graphite.

joint changes.[10] The same type of intra-articular joint change results from the wear and tear of increasing age and in consequence marked changes are demonstrable in most individuals past the fourth decade of life, even though they may never have complained of joint symptoms or joint disease.[11] Therefore, it is apparent that the amount of particulate matter and cellular débris present in a joint may vary considerably from time to time and in consequence the total number of synovial fluid nucleated cells, particularly the mononuclear type of phagocytic cells, must vary also. The minor insults that a joint is subjected to evidently do not cause sufficient intra-articular reaction to result in an increase in polymorphonuclear leukocytes.

A word of warning may be given to anyone interested in studying normal synovial fluid cytology, namely that there are certain species differences which one must consider if he wishes to compare the findings of one species with those of another. These differences concern the total number of nucleated cells as well as the percentage number of any one type of cell. These are best illustrated in the short summary table given below.

The above mentioned data should aid materially in interpreting the cytological variations one observes [1] in normal human synovial fluid.

Summary and Conclusions

1. A study of the cytology of the synovial fluid from the astragalotibial and carpometacarpal joints of young beef cattle showed variations in the total number of nucleated cells and in individual cell types. The widest variations in cell types were observed in the astragalotibial joint fluids.

2. The phagocytic cells (practically all were of the mononuclear type) averaged 57 per cent in the astragalotibial joint fluid and 74 to 78 per cent in the carpometacarpal joint fluid.

3. The non-phagocytic cells (chiefly lymphocytes) averaged 40 per cent in the astragalotibial joint fluid and 20 to 23 per cent in the carpometacarpal joint fluid.

4. The variations in the total number of nucleated cells and individual cell types in these two joint fluids are best explained by the increased amount of débris in the carpometacarpal joint resulting from the articular cartilage defects present.

5. The addition of a small amount of graphite to the synovial fluid before doing the supravital differential cell count aids one in distinguishing between phagocytic and non-phagocytic cells.

6. The wide variations in individual cell types observed in the astragalotibial joint fluid are evidently within the limits of so-called normal. The "normal" figure depends upon the degree of wear and tear, minor trauma, and so on, to which the joint has recently been subjected. Evidently irritations of this grade are sufficient to increase the total number of nucleated cells and the percentage of phagocytic cells exclusive of polymorphonuclear leukocytes.

7. The total number of nucleated cells contained in synovial fluid may increase postmortem but there is very little change in the individual cell percentages.

8. The cellular constituents of normal synovial fluid are not influenced by variations of the blood cytology.

9. There is a definite species difference in the total number of nucleated cells and the percentage of individual cell types contained in normal synovial fluid.

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EFFECT OF CENTRIFUGATION ON HERPETIC INTRANUCLEAR INCLUSIONS WITH A NOTE ON CYTOPLASMIC INCLUSIONS OF UNKNOWN ORIGIN IN THE RABBIT CORNEA *

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A conspicuous evidence of some virus diseases is the production of intranuclear inclusions. It has not been possible thus far to determine unequivocally by the usual microscopic methods whether the smallest visible granules are the virus organisms or the products of a cellular reaction to an invisible virus. The ultracentrifuge developed by Beams, Weed and Pickels [1] provides a new method of approach to these problems in that sufficient centrifugal force is attained to displace the established relationship of cellular elements, and thereby enables one to make comparisons of this displacement with chromatin and nuclear sap, substances about which something is already known.

METHOD

Herpes virus, H. F. strain, originally obtained from the Rockefeller Institute for Medical Research through the kindness of Dr. T. M. Rivers, was employed. Fresh brain of rabbits previously inoculated intracerebrally was triturated and applied to the scratched cornea of rabbits. About 30 to 48 hours later the infected eye was removed and the cornea cut into three pieces, one of which was placed in a duralumin rotor of the ultracentrifuge and whirled at 65 to 70 pounds air pressure for 45 to 60 minutes. The remaining two pieces were fixed in Zenker's acetic fixing fluid, one before centrifugation began and the other after it had been completed. Autolytic effects upon the corneal tissues due to time alone were thus differentiated from centrifugation effects. Corneal tissues, not infected, were treated in the same way. Part of this control material was scratched with a needle about 30 hours previous to removal in order to distinguish factors involved in regeneration from those due to infection

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The preparation of the manuscript was completed while one of us (A.M.L.) was at the Anatomical Laboratory, Washington University, St. Louis, Mo.

when these tissues were centrifuged. Delafield's hematoxylin with eosin, Ehrlich's hematoxylin with triosin, and Giemsa stains were used.

Observations

The cornea is from 4 to 5 cells thick: the basal layer contains large oval nuclei with the long axes perpendicular to the basement membrane, the overlying nuclei are smaller and denser and their long axes parallel to the surface.

Normal Epithelial Cells: When normal corneal epithelium or regenerating corneal cells are centrifuged, both nuclei and cytoplasm are modified to some extent. The cytoplasm balloons centripetally in the outer two or three rows of flattened cells (Fig. 6). The position of the nucleus in the cytoplasm does not change. The chromatin of the nucleus becomes massed toward the centrifugal pole and the opposite end becomes correspondingly free of it but is filled with clear, homogeneous, non-staining nucleoplasm.

No difference was noted in the reaction of nuclei in normal uninjured epithelium and in that undergoing regeneration.

Infected Epithelial Cells: The infected corneal cell nuclei have the appearance represented in Figure 3. The granular inclusion body lies in the center of a nucleus and is usually surrounded by a clear halo. The chromatin is closely packed against the nuclear membrane.

The translocation of chromatin materials by centrifugation in the normal nucleus is relatively slight (Figs. 6 and 7A), but is readily accomplished in the infected nucleus (Figs. 1, 2, and 7B). In the latter the chromatin, as in the normal nucleus, moves toward the centrifugal pole, the fluid, non-staining material forms a narrow middle zone and the inclusion body presses centripetally against the nuclear membrane.

It was sought to determine under high magnification whether the chromatin passes around or through the inclusion body. Most frequently it follows the contour of the nuclear membrane but occasionally, as in Figure 7B, it passes through the mass of inclusion granules and in some cases numerous chromatin particles were found among the granules.

Occasionally the nuclear membrane breaks (Fig. 7C), in which case the granules of the inclusion become slightly separated and pass into the cytoplasm in a centripetal direction. The resistance of the

nuclear membrane against rupture has been noted by Němec,[2] and Luyet and Ernst [3, 4] in plant cells.

Cytoplasmic Inclusions of Unknown Origin: Spherical, cytoplasmic inclusion bodies were found in the epithelial cells of 8 out of 10 corneas examined; the 2 negative cases are doubtful because sufficient material was not available. They are present in control and virus-infected tissues, in both uncentrifuged and in centrifuged portions. Their distribution is not uniform; the bodies are limited to small areas, irregularly scattered, and in any one location the number of inclusions varies greatly. They are present usually in the cells lying above the basal layer and extend into that layer only when very numerous (Fig. 4).

The corneal inclusion body lies closely associated with the nucleus and profoundly changes its shape and character (Figs. 4 and 5). It is bounded by a distinct membrane, inside of which are small, poorly defined non-refractile granules which form part of a reticular network. The granules and reticulum stain with acid dyes but somewhat more lightly than the adjacent cytoplasm. A clear, non-staining fluid fills the interstices.

The inclusion bodies vary in size from 1.5 μ to 10.5 μ. Each one, irrespective of size, lies within the clear, non-staining area which separates nucleus from cytoplasm.

The origin of the inclusion bodies is not known; the smallest bodies thus far identified already occupy the same close proximity to the nucleus as the larger ones (Fig. 5A). Their number in a cell varies from one to four and they lie on either side or end of the nucleus. The effect on the nucleus is the same in each case, namely, a depression of its wall within which the inclusion body extends. The small spheres produce only a slight indentation (Figs. 5A and 5B), where those which are larger in proportion to the nucleus (Figs. 5C and 4A) may nearly constrict it in two parts or may flatten the nucleus until it finally appears as a deeply staining, pyknotic crescent on one side of the inclusion; or in the case of two or more bodies it may be crowded into a small, crumpled, irregularly shaped mass compressed into the space available between the spheres (Fig. 4B). Due to the plane of section the cytoplasmic inclusion sometimes resembles an intranuclear inclusion (Fig. 5D). When they are large and numerous the cytoplasm and the tissue as a whole, as well as the nuclei, appear abnormal.

Effect of Centrifugation on the Corneal Cytoplasmic Inclusion Bodies: Whirling the corneal tissue at 65 to 70 pounds pressure for from 45 minutes to an hour, which is sufficient to displace the intranuclear inclusions of herpes, does not displace the cytoplasmic inclusions in relation either to the rest of the cytoplasm or to the nucleus.

Discussion

Centrifugation separates cellular elements into strata and thereby aids in an analysis of heterogeneous structures. The herpetic intranuclear inclusion is lighter than the nuclear sap or the chromatin; therefore, if the inclusion body arose by abnormal multiplication of elements, which were already present in the normal nucleus, one might expect that this material, being the lightest in the cell, would appear at the centripetal pole of the centrifuged normal nucleus. No such inclusion body precursor is revealed. Its absence, of course, does not preclude the possibility that some element, either chromatin or nuclear sap, is transformed into inclusion body material by the action of the virus, these materials being consumed in the process and consequently decreasing in amount. Evidence for this point of view is weakened when by centrifugation it is apparent that the supposed decrease in chromatin is not as great as it seems to be when marginated (Figs. 1, 2 and 7). Although no quantitative measure has been devised, it appears from the slides and illustrations that when all the chromatin of the infected nucleus is brought to one pole the amount is only somewhat less than that present in centrifuged normal nuclei. Comparison, of course, must be made between nuclei at corresponding levels in the epithelium.

Possible physical antagonism between chromatin and inclusion material, suggested by the tendency of basophilic substance to marginate soon after the appearance of inclusion granules, finds some support under conditions of centrifugation. Examples have been noted in which the chromatin mingles with the inclusion material while passing to the centrifugal pole. However, high magnification of one of these, Figure 7B, shows a narrow clear column surrounding the line of migrating chromatin separating it from the adjacent inclusion material, indicating that a repellent force between the two substances is still operating even when they are forcibly mingled together.

The physical constitution of the infected and normal nuclei is dif-

ferent in that the same centrifugal force readily brings all the chromatin to one pole in the inclusion-bearing cells and only partially pulls the chromatin of normal cells away from the centripetal end of the nucleus. In most instances the chromatin of the normal nucleus is not moved at all. A specific gravity of chromatin greater than that of the nuclear sap has been found in both plant and animal cells (Němec,[2] Beams and King,[5,6,7] Luyet and Ernst,[3,4] and Scott[8]). A wide variation in the amount of centrifugal force necessary to produce the translocation of materials agrees with observations by Beams and King[7] on nerve cells.

The inclusion body itself shows no stratification of materials or separation of granules from the fluid which surrounds them. This is to be expected since the granules are apparently already packed as closely as possible.

Earlier characterization of intranuclear inclusions of virus origin by their acidophilic staining affinities indicated a difference from the basophilic staining chromatin. The existence of a difference between the two materials has been further emphasized by the Feulgen thymonucleic acid reaction which is positive for mammalian chromatin and is negative for inclusions of herpes,[9] virus III,[9] and yellow fever.[10] Likewise, following microincineration, little or usually no mineral ash remains in the inclusions of submaxillary gland virus of guinea pigs,[11] of yellow fever,[12] or of well developed herpetic inclusions,[13] whereas an abundant ash is present from the chromatin. Centrifugation reveals, likewise, a difference, namely that the inclusion material is lighter than any part of the normal nucleus; all of which suggests that the intranuclear inclusion is probably not derived from chromatin.

The corneal cytoplasmic inclusions described do not arise as a result of herpetic infection since they occur equally often in apparently normal rabbits. Their high incidence makes it probable that they persist for relatively long periods of time. Whether they are pathological is not certain but when by their great number and size they distort the nucleus and cytoplasm, as shown in Figure 4, it is difficult to regard them as normal cell structures. The distinct membrane which surrounds each sphere separates it from the adjacent cytoplasm, but the internal structure is not greatly different. Their principal effect is on the nucleus but the resulting modification on its shape and character is not proof of a detrimental influence. The in-

dentation of the nucleus calls to mind a similar reaction by vaccine virus inclusions in fixed and stained preparations. The shrinkage space which surrounds the nucleus and its apposed corneal cytoplasmic inclusion body may be indicative of a perinuclear material different from that existing in the remainder of the cytoplasm. This agrees with a suggestion by Cowdry,[14] in his study of vaccine virus inclusions, that the "appearance is strongly suggestive of shrinkage at interfaces between fluids of different consistency and composition."

No leukocytic infiltration occurs in the region where the corneal inclusions occur. Leukocytic infiltration in vaccinia is variable; in some cases it may, likewise, be absent from the region in which the inclusion bodies are found.

Summary and Conclusions

1. Centrifugation of rabbit cornea inoculated with herpes virus concentrates the marginated chromatin to the centrifugal pole, causes the nuclear sap to form a clear stratum across the middle of the nucleus, and moves the inclusion to the centripetal pole.

2. The concentrated marginated chromatin, when brought to one pole, appears to be slightly less in amount than the chromatin of the normal nucleus.

3. Antagonistic forces between chromatin and inclusion body, expressed by the phenomenon of margination, is still operating when chromatin is forced by centrifugation through the inclusion body, in that the chromatin in passing through is separated from the granules by a distinct space.

4. Centrifugation of normal nuclei moves the chromatin slightly toward the centrifugal pole and the nuclear sap to the opposite pole. No substance is concentrated which by its relative specific gravity or staining can be regarded as the direct antecedent of the granules present in the herpetic inclusion body.

5. Cytoplasmic inclusions of unknown origin were found in the epithelial cells of both normal and infected rabbit corneas. They indent the nuclear walls and when large, the nuclei become crescentic or compressed to a small, irregularly shaped body. The pathological nature of the corneal cytoplasmic inclusion body is not yet established.

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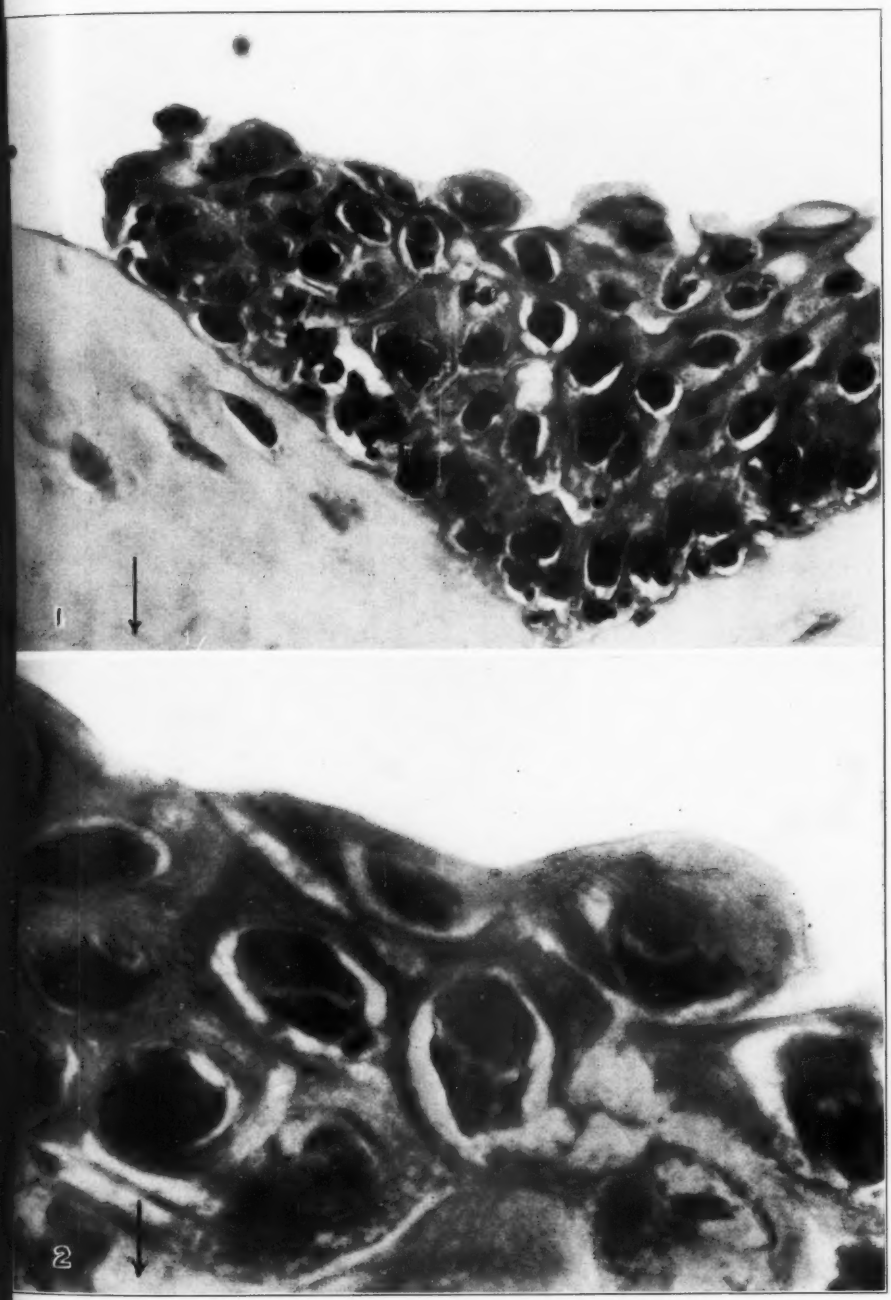
DESCRIPTION OF PLATES

The photomicrographs were taken by F. W. Kent. The drawings * were made by Miss G. L. Larsen. The arrow in Figures 1, 2, 6 and 7 points to the centrifugal pole.

PLATE 127

FIGS. 1 and 2. Photographs of the same group of corneal cells in which the intranuclear inclusions of herpes have been displaced centripetally by centrifuging for 45 minutes at approximately 494,000 times gravity. × 850 and × 2640.

* We are indebted to the Graduate School of the University of Iowa for funds for the preparation of the drawings.



Lucas and Herrmann Herpetic Intranuclear Inclusions

PLATE 128

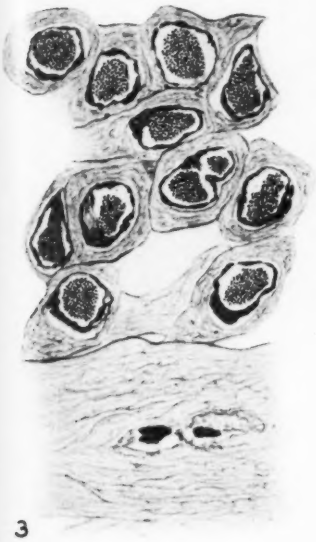
FIG. 3. A control section from the same cornea as shown in Figures 1, 2 and 7, showing cells containing intranuclear inclusions of herpes. × 1300.

FIG. 4. A region of cornea containing numerous cytoplasmic inclusion bodies which are of unknown origin. × 1300.

FIG. 5. Four cells showing the relation of cytoplasmic inclusions to the nuclei. × 1300.

FIG. 6. Normal corneal cells after centrifuging for one hour at approximately 494,000 times gravity. × 1300.

FIG. 7. Drawing of the same group of cells illustrated in Figures 1 and 2. × 1300.



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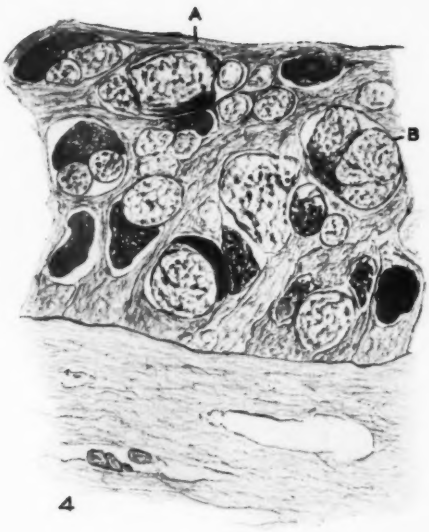

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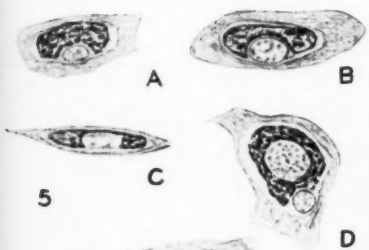

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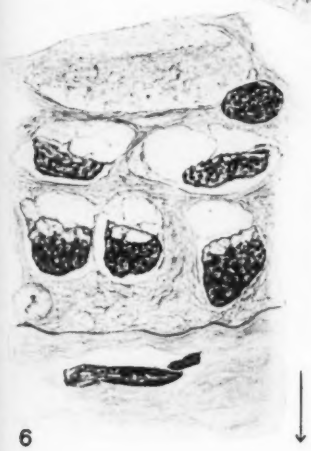

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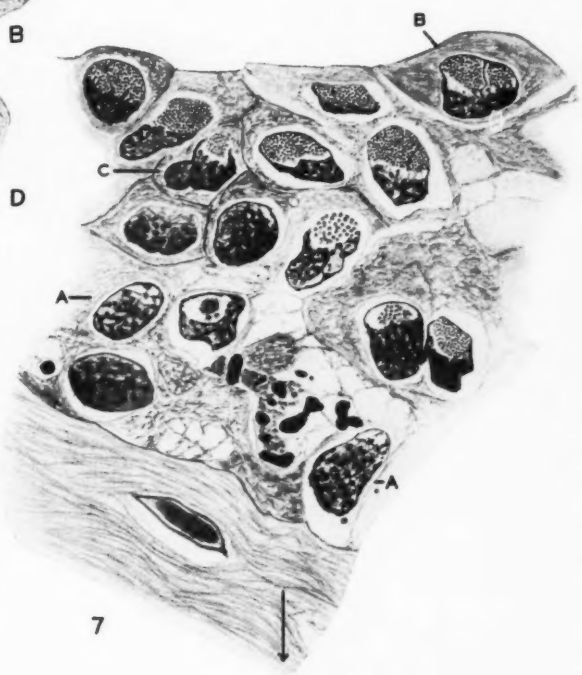

7

Lucas and Herrmann

Herpetic Intranuclear Inclusions

PRIMARY AMYLOIDOSIS LIMITED TO TISSUE OF MESODERMAL ORIGIN *

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DEDICATED TO PROFESSOR ANTON GHON OF THE GERMAN UNIVERSITY IN PRAGUE IN HONOR OF HIS SEVENTIETH BIRTHDAY

Amyloidosis secondary to various chronic diseases is relatively common and well known, in contrast with the type regarded as atypical in which the condition appears to be primary. About 35 cases of the latter type have been reported, chiefly from German sources. Of these there are 17 which resemble the type described here. Most of them were studied and reported within the past 6 years.

The following case is of unusual interest since it is apparently the first reported to be diagnosed clinically and because of the generalized and extensive involvement of the smooth musculature of the cardiovascular system, the mesodermal structure of the lung and serous membranes.

REPORT OF CASE

Clinical History: In January, 1933, a female, 41 years of age, first noted intermittent pains in the shoulders and arms, especially at night, aching pains in the legs after walking, and later general weakness. A goiter appeared about the same time. In May, 1933, sore throat developed with cervical adenitis, fever and pain, which necessitated 2 weeks stay in bed. Because the fever persisted the patient was sent to a hospital by her physician. An X-ray examination of the chest, various agglutination tests and a Mantoux test all gave negative results. There were, however, albumin (1+) and a few red cells in the urine, and the hemoglobin was said to be 60 per cent. During the summer and autumn of 1933 weakness persisted, edema of the legs and ankles was noted toward evening and there was continual low grade fever, tachycardia and malaise. The record is then blank until July, 1934, when submental swelling was noted. The menstrual periods had been scanty during the previous year and ceased in September, 1934. In October the tongue was noted to be thick and red and felt as if blistered. Weakness, dyspnea on exertion, loss of weight, dysphonia and dysphagia increased. The tongue continued to increase in size and the skin over the chin became hard. The condition was regarded as malignant. Because of the progressive nature of the disease and loss of 31 pounds in weight, the patient entered the University Hospital in January, 1935.

* Received for publication July 1, 1935.

On physical examination the patient evidently had lost much weight and there was dyspnea and a slight icteric tinge of the skin. The veins of the neck were distended and the skin over the chin was thickened, waxy and fixed to the underlying tissues. Firm, large, tumor-like masses were present under the mandible and the posterior cervical lymph nodes were enlarged and firm. The thyroid gland was diffusely enlarged. The tongue was thickened to about twice the normal size, with a red smooth surface indented by the teeth. There was evidence of pleural effusion and râles were present in the left lung. The heart appeared to be normal. The systolic blood pressure was 128 and the diastolic 78. The liver was palpable but apparently normal. Examination of the pelvis revealed hard, leathery induration of the clitoris and vulva, thickened, firm vaginal walls, a hard cervix and an enlarged firm uterus with irregular contour and restricted mobility.

Laboratory Data: Urine normal, no Bence-Jones protein; red cells 5 million per cmm., hemoglobin 78 per cent, leukocytes 4900 per cmm. with 77 per cent polymorphonuclear cells. Blood Wassermann reaction negative; basal metabolic rate +12 per cent; sedimentation rate first hour 48 mm., second hour 77 mm. The Congo red test was negative as 30 per cent of 125 mg. were removed from the blood in 1 hour. Plasma proteins per 100 cc.: fibrinogen 0.52 gm., euglobulin 0.38 gm., pseudoglobulin 1.9 gm. and albumin 3.52 gm., giving a total of 6.32 gm. Mantoux test 0.1 mg. positive.

X-ray examination (Fig. 1) revealed pleural effusion in both bases, more in the left, and a diffuse infiltration throughout both lungs following the bronchovascular trees and suggesting some type of pulmonary congestion of rather extreme degree or an infiltrating process. There was a large mass in the region of the righ hilum. The femurs, pelvis, humeri and skull were negative.

Clinical Course: The temperature ranged between 37.2° C. and 37.8° C. (99° F. to 100° F.) until January 17th when peritonitis developed, characterized by chilly sensations, emesis, abdominal distention, fever of 39.7° C. (103.4° F.) and 56,000 leukocytes per cmm. Death occurred 6 days later.

A diagnosis of amyloid disease of the tongue, skin and probably of the genitalia and mediastinum, with terminal peritonitis, was made. The diagnosis was confirmed by biopsy of the tongue, skin and vaginal wall. Previous experience and the privilege of studying another case with an amyloid tongue which was subsequently reported by Michelson and Lynch [1] led to the clinical recognition of the case here reported.

Postmortem Examination

The right pleural cavity contained a liter of clear fluid, the left was partly obliterated. There were no adhesions in the pericardial sac. The heart weighed 360 gm. The left auricular wall when cut did not collapse and appeared as if frozen or infiltrated with carcinoma. It was rubbery, and when bent sprang back into position. The right auricle was less involved, while the ventricles, chordae tendineae and the root of the aorta appeared to be normal in color and consistence.

The right lung weighed 1160 gm., the left 550 gm. There were edema and patchy lobular atelectasis, most marked on the left. The consistence was unusual and suggested that of frozen lungs; they were firm, heavy and did not collapse. Apparently they were not consolidated and on the cut surface the alveoli remained open. The pulmonary veins were from 2 to 4 mm. thick, stiff and fixed to the mediastinal structures. The entire mediastinum was firm, hard and fixed. The heart was held firmly in the mass by the greatly thickened vessels. The trachea and aorta were similarly fused together as if by fibrous tissue, although nothing was found to suggest mediastinitis. The lymph nodes were normal. The wall of the lower portion of the esophagus was thickened, stiff and did not collapse. There were no abnormal changes in the aorta. The tongue appeared as described clinically, with its epithelial surface intact. The submaxillary lymph nodes were not enlarged. Enlargement of the thyroid gland (150 gm.) was due to multiple adenomas.

The peritoneal cavity showed generalized peritonitis with injected serous surfaces and turbid fluid containing fibrin and hemolytic streptococci. The spleen (325 gm.) was uniformly dark in color, resembling the type encountered in long-standing passive congestion. A portion tested with iodine and sulphuric acid failed to give the reaction characteristic of amyloid substance. The liver (2250 gm.) was not remarkable and failed to give the amyloid reaction.

The kidneys (490 gm.) showed "flea-bitten" surfaces characteristic of acute glomerulonephritis or of embolic nephritis. The hemorrhagic appearance was also found on the cut surfaces. The right ureter was normal but the lower third of the left was thickened. Its wall and adjacent tissues, including the ovarian vein, were involved in an acute inflammatory reaction. This process extended through the left broad ligament into the region of the cervix. Edema, small pockets of pus and the presence of many hemolytic streptococci indicated that this was a terminal infection.

The uterus was enlarged to the size of a 2 months gravid uterus, the muscle averaging 4 to 5 cm. in thickness. It cut with increased resistance. Both cervix and uterus were hard. The vaginal wall was remarkably thickened and difficult to cut. Its appearance and consistence resembled that of a malignant infiltration. The ovaries were hard, sclerotic and atrophic. There was an acute pyophlebitis of the left ovarian vein.

No important changes were noted in the stomach, bowel, pancreas, adrenals, urinary and gall-bladder, brain or meninges.

Anatomical Diagnoses: Amyloidosis of the tongue, heart, lungs, esophagus and pelvic organs; acute generalized peritonitis; pulmonary edema and atelectasis; pleural effusion; phlebitis of the left ovarian vein; glomerulonephritis or embolic nephritis; and adenomas of the thyroid gland.

Microscopic Examination

The sections were stained with hematoxylin and eosin, Mallory's connective tissue stain, azocarmine, Van Gieson's stain, methyl violet, cresyl violet, Congo red, and iodine followed by sulphuric acid. Unless stated otherwise, the descriptions are based on the appearance of sections stained with hematoxylin and eosin.

Except for the changes incident to the terminal infection, namely phlebitis of the cervical, parametrial and ovarian veins and peritonitis, the findings centered on the amyloid deposits and connective tissue changes. The blood vessels throughout the body showed marked deposition of amyloid substance. The increase in size of the vessel walls and the resulting distortion of the tissues made it difficult in places, especially in the lung, to distinguish between veins and arteries. In the cervix and tongue the distinction was fairly clear; in the liver, kidneys and heart the identification was easy. As far as could be determined, the changes observed were entirely limited to the arteries, principally the small and medium sized vessels. This vascular involvement was present in the subcutaneous tissue, fat, loose areolar tissue, peripheral muscle, thyroid, salivary glands, larynx, lungs, heart, esophagus, small bowel, liver, spleen, adrenals, kidneys, uterus, cervix, ovaries, vagina, bladder and pancreas. The arterioles, such as those entering the glomeruli of the kidneys, and the capillary vessels throughout the tissues were normal. In the largest vessels, such as the aorta and renal arteries, only the vasa vasorum were infiltrated. The degree of involvement was not uniform in all the vessels. Those of the tongue, cervix, vagina and submucosa of the small bowel were much more extensively affected than those of other tissues.

In detail, the wall of the involved vessel was enormously thickened (Figs. 2, 3 and 4), three fairly definite layers could be distinguished — a loose cellular internal zone, a compact relatively acellular or

hyaline-like medial layer, and a fibrous, compressed peripheral layer. The internal zone was made up of a loose network of fibroblasts with a layer of endothelium separating it from the lumen. The peripheral layer appeared as though it were the remains of the adventitia compressed by a greatly enlarged media. In the middle layer only a few irregularly grouped nuclei were seen. Among these nuclei were masses of homogeneous, eosin-stained substance. Further analysis of these tissue changes was made by means of the differential stains as described below.

The size of the lumens was difficult to estimate. The compression of the adventitia and the loss of substance between the vessels indicated that the vessels enlarged peripherally. An estimation of the ratio of the diameter of the lumen to that of the entire vessel, therefore, was of no value. The lumen was seldom occluded. The absence of patchy atrophy of the kidney such as is seen in sclerotic disease of arteries also indicated that very little, if any, occlusion took place.

In addition to the arterial changes there were connective tissue changes in the tongue, esophagus, auricles and ventricles. The muscle fibers were spread apart by varying amounts of irregularly distributed material, partly fibrillar and partly homogeneous (Figs. 5 and 6). In certain places this material was in wide bands. The muscle bundles were not themselves invaded but suffered atrophy and replacement, as evidenced by loss of striations, shrinking of the bundles and, finally, complete atrophy. In the tongue and auricle wall the replacement of muscle took place in a coarse and irregular fashion; in the esophagus and ventricle it was fine and evenly distributed.

The lung manifested still another change (Fig. 7). In addition to the heavily involved blood vessels there were small bands, rounded nodules and irregular plaques of eosin-stained material within the alveolar walls. None of the deposits was large enough to fill the alveolar space. They were uniformly distributed throughout all portions examined from both lungs with scarcely a single microscopic field free from involvement. The changes accounted in part for the unusual roentgenogram of the lungs and the unusual consistence noted in gross. The bronchial mucosa, muscle and lymph nodes were not involved.

A similar eosin-stained substance was found in the serous mem-

brane of the spleen, accessory spleen and small bowel, but not in that of the examined sections from the liver, ovary, uterus, lung or heart. The thickening was uniform and regular, and was differentiated from simple, thickened fibrous peritoneum by the absence of nuclei and by the use of special stains.

The parenchyma of the liver, spleen, adrenals, pancreas and thyroid, other than the blood vessel changes, showed no significant change. The glomeruli of the kidneys showed a terminal glomerulitis, manifested by slight proliferation of the endothelium. At the periphery of several of the arteries in the cervix there were large giant cells. The nuclei of these cells were irregularly arranged in the center of the cytoplasm. No inclusions or phagocytosed granules were visible. A few small lymphocytes were scattered about the giant cells. This collection of multinucleated cells was not found in other parts of the body. It may have represented a foreign body reaction to the presence of the amyloid.

Special Staining Methods

The similarity of staining reactions of the substance, as found in the arterial walls and elsewhere, permits inclusive description of the study. Of the stains used, Mallory's connective tissue stain proved to be of most value. It was evident that the deposits were composed of two different materials. One was an irregularly arranged, rough, coarse substance which stained like typical amyloid substance with Mallory's and Van Gieson's stains, eosin, cresyl violet and methyl violet, but failed to stain with Congo red or with iodine and sulphuric acid. This amyloid substance appeared to be embedded in a different and more delicate appearing substance, which stained less deeply with eosin and did not give the amyloid reaction (Fig. 5). The amyloid substance was found only within the largest collections of the latter material. In the ventricular muscle, for example (Fig.6), there was extensive strangulation of the fibers by fine strands of the delicate appearing substance in which only occasional faintly stained areas suggested the presence of typical amyloid substance. On the other hand, under the intima of the auricle, there were wide bands of the delicate substance and these contained numerous rough masses of amyloid substance. The atypical, delicate appearing material which formed the bulk of the deposits between the muscle bundles was regarded as "ground" substance.

Azocarmine is generally believed to stain both adult collagen and its immature or precollagenous form, whereas by Van Gieson's and Mallory's methods only the adult type is stained. The ground substance in question did not react like adult collagen in this respect. It did not retain Van Gieson's or Mallory's stain, stained faintly with eosin but, like premature collagen, was stained deep blue with azocarmine. This behavior, however, does not prove it to be precollagen. Since amyloid substance is also stained with azocarmine and eosin, one may suggest with equal right that the ground substance may be the precursor of amyloid substance. The presence in this case of amyloid in only the larger, apparently older masses of the ground substance was so constant as to suggest strongly that the latter material probably was "pre-amyloid." It is possible also that the amyloid substance itself may not be entirely mature in the usual sense since it failed to react typically with Congo red or with the iodine and sulphuric acid test. One is also led to suspect that the "ground" substance increases at the expense of the muscle fibers and, as it increases in volume, amyloid appears in the center or oldest portion. The pathogenesis of this substance appears to be fundamentally different from that found in secondary amyloidosis. In the present case the substance appeared to have been formed and deposited locally whereas in the secondary form it is deposited in organs and areas especially designed for the removal of normal or abnormal excess substances from the circulating blood. The relation of the substance to "hyalin" was widely discussed 30 years ago, but until further chemical studies are made little progress can be made in this regard.

Discussion

Aside from Wichmann's [2] comprehensive survey in 1893 in which amyloid disease was classified into local and generalized forms, no attempts at further classification were made until Lubarsch's [3] paper appeared in 1929. Lubarsch recognized an essential difference between various forms and grouped them into the typical or commonly recognized form and the atypical (systematized) form into which group the present case falls. Since then numerous terms have been introduced, some of which seem to confuse rather than clarify the classification. Terms such as typical, genuine, classical, visceral, generalized, orthochromatic, pericapillary, periglandular and so on

are applied to the common form of amyloid disease which often follows chronic illness. The atypical form is described as primary, unusual, idiopathic, systematized, paramyloidosis, and so on. Several separate groups are proposed to accommodate variations such as tumor-forming amyloidosis, variations in staining properties, and according to the localized distribution of amyloid substance. The terms "typical," "genuine" or "classical" are justified only to the extent that they signify the form of disease first studied and more commonly encountered. The terms "visceral," "generalized," "systematized" or "localized" may apply to either form of the disease. The adjective "unusual," or "paramyloidosis," are pointless and until more knowledge of the chemistry of amyloid protein or proteins is attained, subdivision on the basis of staining reaction is of little value. A simple clinicopathological classification is as follows:

I. Primary amyloidosis
II. Secondary amyloidosis
III. Tumor-forming amyloidosis
IV. Amyloidosis associated with multiple myeloma.

Even this proposal is not entirely satisfactory because of the frequent overlapping of characteristics. For example, von Bonsdorff's case [4] apparently primary with multiple amyloid tumors, resembled the type which accompanies multiple myeloma. In Gerber's [5] case of the "typical" form there was extensive involvement of the bone marrow. In the case reported by Michelson and Lynch,[1] presumably one of multiple myeloma, amyloid substance was distributed as in the primary form, but except for Bence-Jones proteinuria and doubtful X-ray evidence it is, however, uncertain, without autopsy evidence, if myeloma actually existed. In general, however, certain differences are quite constant.

I. *The primary form* is characterized by (*a*) absence of preceding disease, (*b*) no involvement of organs or tissue usually affected in the secondary form, (*c*) involvement of mesodermal tissue, cardiovascular system, gastro-intestinal tract, smooth and striated muscle and lymph nodes, (*d*) variation in staining reactions and (*e*) tendency to nodular deposits. This form is thoroughly discussed by Strauss.[6]

II. *The secondary form* usually follows chronic disease and is

characterized by large deposits, especially in the spleen, liver, kidney and adrenals, and by typical staining reactions.

III. *Tumor-forming amyloidosis* has been especially studied by von Bonsdorff. This form is characterized by the presence of small, solitary or multiple tumors in the eye, bladder, urethra, pharynx, tongue and especially in the respiratory tract. It is usually of the primary type but is distinctive enough to be grouped separately.

IV. *Amyloidosis occurring with multiple myeloma* is in a class apart. It is secondary in nature but the distribution and character of the deposits frequently resemble those of the primary form except that huge deposits may occur in the joints and elsewhere. The spleen and liver are seldom infiltrated. Small deposits are occasionally found in blood vessels of the heart, spleen, and elsewhere. Thirty-seven cases of the latter group have been surveyed by Magnus-Levy.[7]

There have been thus far about 35 cases reported which fall into Group I [8]; 17 of these resemble more or less the case reported here, while in the remainder the amyloid substance was localized to one or two organs, as in the cases reported by Beneke and Bönning, Landau, Beckert, Kann, Beneke, Königstein, Budd, Brocher and Humphreys.[8] Picchini and Fabris' and Gottron's cases were almost identical with ours.

The clinical characteristics of cases included in Group I are fairly characteristic. The disease is apparently primary in nature, occurs in middle-life or later, and is characterized chiefly by involvement of the skin, tongue and smooth and voluntary musculature. The skin, usually of the face and neck is thickened and stiff, the tongue and involved muscles become greatly enlarged and firm. Infiltration of the intestine results in diarrhea or constipation and occasionally hemorrhage. Hemorrhages may occur into the skin and elsewhere. Involvement of the mediastinum, lungs and heart may result in dyspnea or heart failure.

Weakness and loss of weight are frequent but the blood pressure is seldom affected. Death from renal insufficiency, as occurs in the secondary form, has not been reported. The Congo red test was positive in Gottron's case but negative in von Bonsdorff's and our own.

Histologically the characteristics are striking in respect to the generalized involvement of the smooth and striated musculature, especially of the cardiovascular system, gastro-intestinal and genito-

urinary tracts, tongue and diaphragm. Involvement of the central nervous system has not been reported. The media of small or medium sized arteries is chiefly affected.

In muscle tissue amyloid deposits occur between the fibers and in the connective tissue spaces in the form of bands, masses or nodules. Deposits may be found in sebaceous and sweat glands, in the loose connective tissue around the aorta, in tendon sheaths, in the alveolar walls of the lung and elsewhere. With special stains the substance may react like "typical" amyloid substance, but usually atypically, weakly or not at all.

The etiology of primary amyloidosis is unknown. Strauss cited a number of associated diseases, but in practically all cases, as in our own, these appeared to be incidental or terminal. Letterer [9] suggests the involvement of an antigen-antibody reaction but in the reverse sense as compared with the reaction presumed to exist in the secondary form of amyloidosis. According to Wichmann [2] and Schmidt [10] the process in general resembles infiltration more than it does disintegration or degeneration, since the organs involved usually increase in size and weight without evidence of intracellular deposits. In our own case, exclusive and widespread involvement of tissue of mesodermal origin and histological evidence of the local origin of the amyloid substance strongly implicated some unknown change, perhaps in the nature of generalized metabolic perversion of tissue of this particular origin.

Summary

A case of primary amyloidosis of 24 months duration is reported. After an incidental respiratory infection there was continual low-grade fever, progressive weakness, loss of weight and slight evidence of cardiac failure. Swelling of the tongue was noted 15 months later and death was caused by acute peritonitis. A clinical diagnosis of amyloidosis was made and confirmed by biopsy and autopsy. The Congo red test was negative.

The striking pathological change was amyloidosis limited to tissue of mesodermal origin, notably the smooth musculature of the medium sized arteries of all of the organs and tissues examined, the mesodermal structures in the lung and the serosal surfaces.

A simple classification of amyloid disease is presented with special reference to the type regarded as primary in nature.

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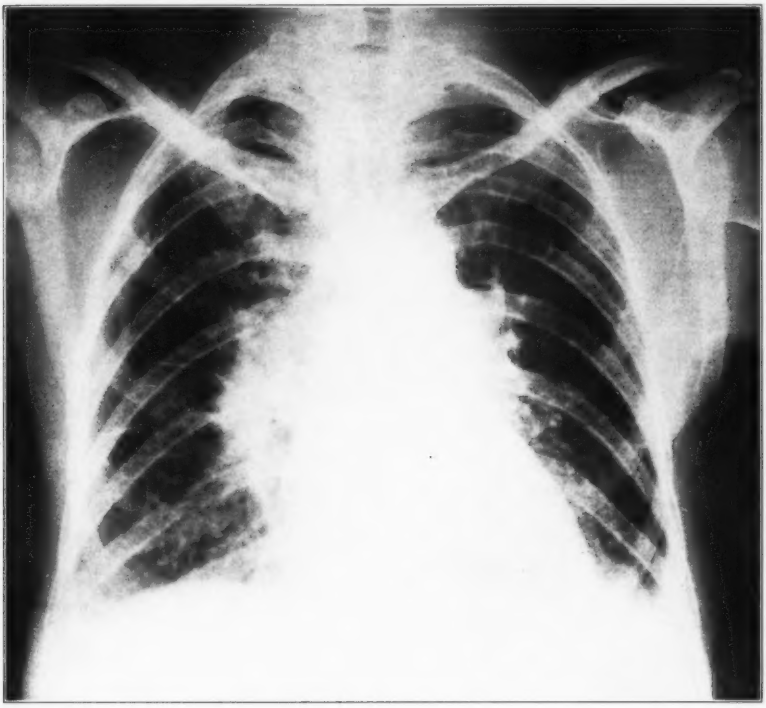
DESCRIPTION OF PLATES

PLATE 129

FIG. 1. Roentgenogram showing diffuse amyloid infiltration of mediastinum and lungs.

FIG. 2. Arteries of cervix. The media is enormously enlarged by amyloid substance which displaces the nuclei irregularly. The intima is a loose network of fibroblasts with a layer of endothelium. The adventitia is partly obliterated, as if by pressure. Hematoxylin-eosin preparation. × 150.

FIG. 3. Arteries of tongue showing changes similar to those described in Figure 2. The muscle fibers are spread apart by amyloid.



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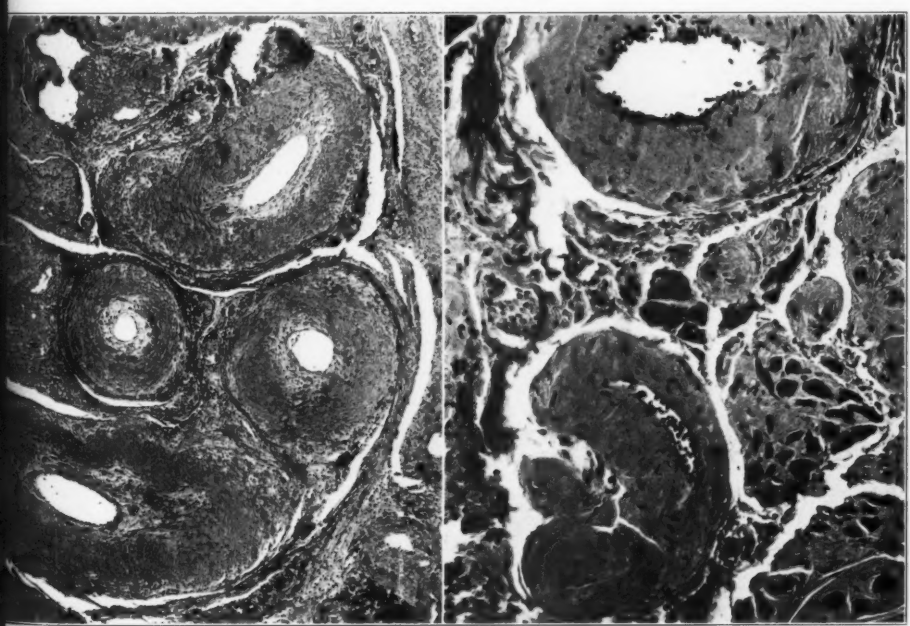

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Reimann, Koucky Eklund Primary Amyloidosis

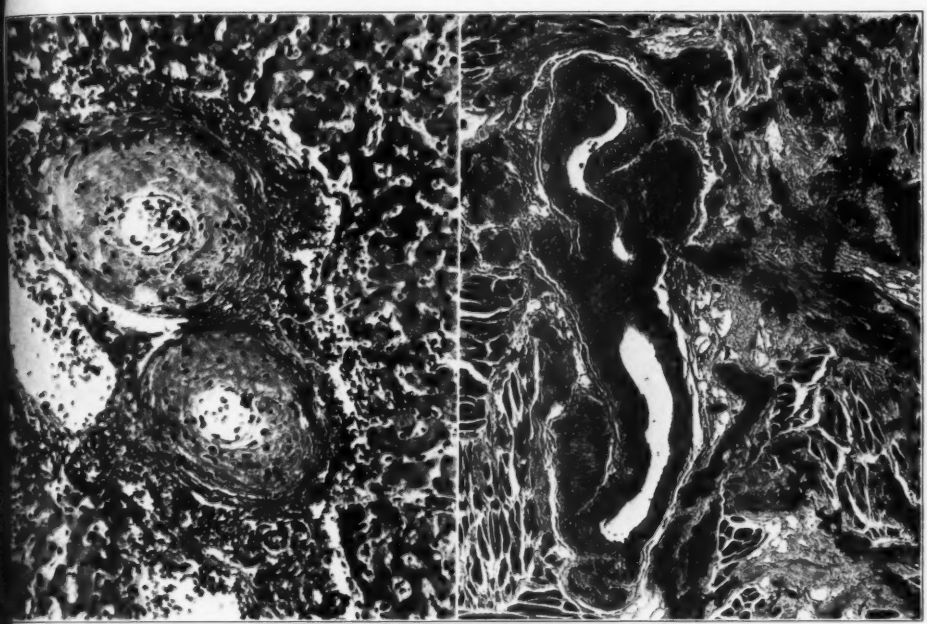
PLATE 130

FIG. 4. Arteries of liver showing same changes as those in Figs. 2 and 3. × 150.

FIG. 5. Tongue. Large masses of amyloid substance replace and displace the muscle fibers. Methyl violet stain. × 60.

FIG. 6. Heart. Left ventricle stained with azocarmine. There is diffuse atrophy of the muscle due to pressure of a substance which stained like immature collagen; in sections stained with methyl violet the larger deposits also gave a faintly positive reaction like amyloid substance. × 250.

FIG. 7. Lung. There are large and small masses of homogeneous material, the central portion of which stained like amyloid substance. The arrows indicate plaques or beads of the substance in the alveolar walls. Hematoxylin-eosin stain. × 300.



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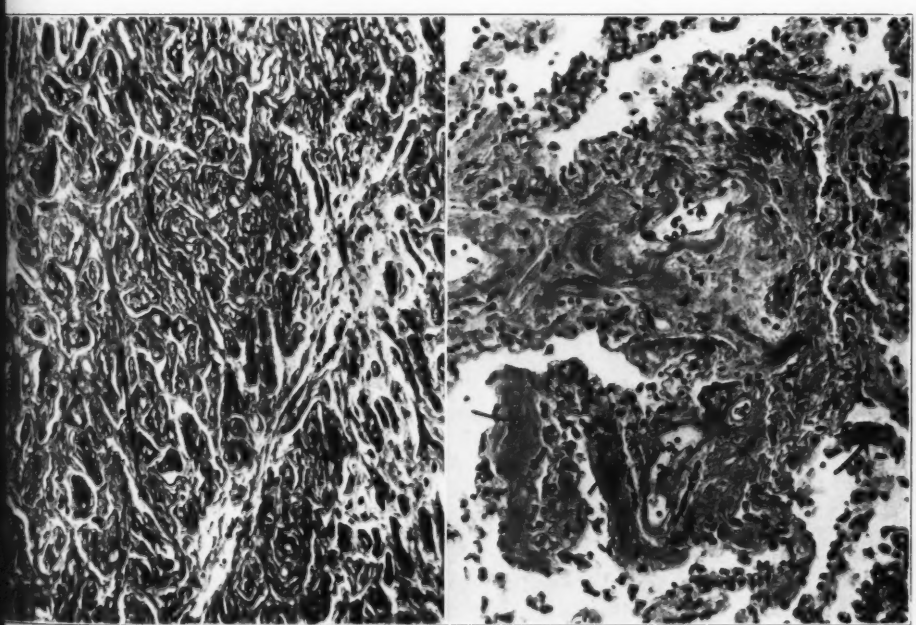

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Reimann, Koucky Eklund Primary Amyloidosis

SPONTANEOUS RUPTURE OF THE PULMONARY ARTERY *

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Spontaneous rupture of the pulmonary artery implies a break in the continuity of the vessel wall which occurs without evidence of trauma, aneurysm, or gross extrinsic pathological changes.

Among the extrinsic causes of perforation of this large vessel are those producing erosion of the wall from the external surface, such as tuberculosis, inflammation, or malignant changes in the surrounding structures. Fittje [1] reported a case of a 34 year old male where death was due to hemorrhage caused by erosion of a necrotic gumma into the pulmonary artery. Clerc, Bascourret and Froyez,[2] Bonte,[3] and Sternberg [4] described ruptures of luetic aortic aneurysms into the pulmonary trunk.

Traumatic ruptures of the pulmonary artery may be caused by crushing of the thoracic cage, stab wounds, gunshot wounds and so on. Marble and White [5] reported death in a young army officer from multiple pulmonary hemorrhages 5 months after a gunshot wound in the chest. Autopsy showed a traumatic aneurysm of the right pulmonary artery with a valve-like opening connected with a bronchus.

Henschen,[6] Posselt,[7] Wahl and Gard,[8] and D'Aunoy and von Haam,[9] in a thorough search of the literature up to 1933 collected 87 cases of pulmonary aneurysm involving some portion of the trunk or its two main branches. In 10 of these cases death was due to rupture of the aneurysm. Two were of the dissecting type. A dissecting aneurysm is not a true aneurysm in the usual sense, but is formed by the escape of blood into the wall of a vessel leading to the separation of the coats. Dissecting aneurysms of the aorta have been reported many times,[10,11,12] and 9 cases have been found in 6800 autopsies by the Department of Pathology of Stanford University. Spontaneous rupture of a vessel may lead to the formation of a typical dissecting aneurysm unless the rip is so complete that death ensues immedi-

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ately. On the other hand, a dissecting aneurysm may be the initiating factor in a complete rupture. These two conditions are similar, are probably the results of the same underlying pathological processes, and the two terms are often used interchangeably.

The occurrence of a spontaneous rupture of the pulmonary artery with slight dissection and fatal hemorrhage into the pericardium is so rare that a search of the available literature reveals only 2 cases [13,14] at all similar to the following.

Report of Case

Clinical History: Lane Hospital No. 167579. The patient was an American widow, 44 years of age, who gave no history of rheumatic fever or chorea. There had been a normal pregnancy at the age of 18. At 28 she developed congestive heart failure with ascites and edema. After 6 weeks bed rest she continued to work for 9 years. Another brief period of failure occurred at 37, and a more severe one at the age of 38 years, when she was brought into Lane Hospital. Her condition during this attack was reported because of the development of a curious heart rhythm.[15] Although continually disabled, she did not have medical care from her 38th until her 44th year, when she was again brought into the hospital, stuporous, deeply cyanotic, with severe dyspnea and markedly distended neck veins. Ascites and liver enlargement had been present for years. On her first hospital entry the liver was large and pulsating, but 6 years later it was hard and easily movable. She improved during 15 days in the hospital but died in her sleep.

Physical Examination: The heart was huge, and by X-ray had a "mitral" shape, with slight dilatation of the aorta, and a prominent pulmonic arch. The rhythm was regular except for the episode occurring during her first hospital entry.[15] There was a loud diastolic murmur at the apex and in the second and third left interspaces close to the sternum; no systolic murmur was present. The apex beat moved with respiration and change of chest position. The blood pressure was 130/90. Although the neck and facial veins were distended, they pulsated very little. The absence of orthopnea over a 6 year period in this deeply cyanosed patient with a huge heart, large liver and distended veins was most remarkable, as also was her continued activity for 16 years after the first onset of congestive failure.

Laboratory Data: The electrocardiogram was that of right axis deviation with P waves broad and notched. Blood, urine and Wassermann tests were not remarkable.

Diagnosis: Mitral stenosis. Tricuspid insufficiency was suggested by several examiners, but none mentioned tricuspid stenosis.

Autopsy Report

An autopsy (No. XXXVI–397) performed 12 hours after death showed the body of a small, well developed, poorly nourished female of about 45 years of age, weighing about 100 pounds. Livor mortis

was marked, as was cyanosis of the head, neck and shoulders, with the neck veins distended to 2.25 cm. in width. A moderately soft nodule 3 cm. in diameter was palpable on the right side of the neck apparently attached to the thyroid gland. The chest was flat and symmetrical. The abdomen was moderately distended with fluid. The extremities were wasted and free of edema.

Both pleural cavities were free of adhesions, but the left contained 200 ml. and the right 1500 ml. of thin, slightly bloody fluid. The striking finding was the pericardium distended with 1000 ml. of dark clotted blood. The heart chambers were collapsed, except for a markedly distended right atrium filled with clot similar to that in the pericardium. The venae cavae were each dilated to 4.5 cm. in diameter and filled with clot. The pectinate muscles of the right atrium were hypertrophied to 0.6 cm. in diameter. The tricuspid valve measured 10 cm. in circumference about the base, but the cusps were moderately thickened with fibrous tissue and adherent, forming a rigid, circular stenotic opening 1.5 cm. in diameter and 5 cm. in circumference (normal 12 cm.). The right ventricle was markedly dilated and formed half the apex; the muscle was firm and hypertrophied to 0.9 cm. at the base, with large papillary muscles 1 cm. in diameter. The pulmonary valve was normal, 7 cm. in circumference. The pulmonary artery measured 9 cm. in circumference 1 cm. above the valve; the wall was 0.15 cm. thick and the intima was roughened by scattered atheromatous plaques, the largest 1.2 cm. in diameter. The left branch measured 7.5 cm. in circumference and the right 6.5 cm. with similar atheroma. *In the left lateral wall of the pulmonary trunk was a fresh, jagged, longitudinal separation of the intima and a portion of the media measuring 7 cm. in length extending from the base of the valve upward toward the left branch through one of the atheromatous patches* (Fig. 1). Two cm. above the valve the rupture was complete with a 1 cm. opening through the adventitia and pericardium. Here the adjacent tissues were slightly hemorrhagic. The lateral separation of the media extended only 1.5 cm. from the tear in its widest dissection. The whole appeared to be fresh as though the result of an instantaneous rupture. The left atrium was moderately dilated and the muscle hypertrophied to 4 mm. The mitral valve measured 7 cm. about the base, the cusps were adherent and fibrous, forming a rigid circular orifice 2.5 cm. in circumference (normal 10 cm.). The left ventricle was normal in

size, muscle slightly flabby, 1 cm. in thickness at the base, with no visible scarring. The aortic valve was normal, 6.5 cm. in circumference. The aorta measured 7.5 cm. in circumference 1 cm. above the valve, with slight atheroma at the base, but the arch was normal. There was also slight atheroma of the thoracic and abdominal aorta and of the coronary arteries. The heart with short lengths of the great vessels weighed 435 gm.

Both lungs contained small apical scars. The right was almost completely collapsed. The left was pink and air-bearing. Neither showed a noticeable increase in fibrous tissue. The large branches of the pulmonary artery were moderately atheromatous.

A moderately soft, globular, well encapsulated tumor 2.5 cm. in diameter projected laterally from the right lobe of the thyroid gland.

The peritoneal cavity contained 2000 ml. of clear, pale yellow fluid. The abdominal viscera were markedly congested. The spleen weighed 450 gm. The left kidney was hypoplastic, measured 7.5 by 2.5 by 2.5 cm., and weighed 35 gm. The right kidney measured 13 by 5.5 by 4 cm. and weighed 210 gm. Both showed old flat infarct scars. There was a slight cervical erosion and a small subserous fibromyoma of the uterus. In addition to marked congestion the gastro-intestinal tract revealed a healing puckering ulcer in the midportion of the lesser curvature of the stomach, and dark brown pigmentation of the large bowel (melanosis coli). The liver measured 25 by 18 by 8 cm. and weighed 1650 gm. The surface was roughened by numerous nodules varying from 0.3 to 3.5 cm. in diameter and cut with considerably increased resistance. The cut surface showed a marked passive congestion and atrophy with extensive fibrosis, also dilatation of the portal veins. The adrenals and pancreas were normal.

Microscopic Examination

Histological sections of all organs were examined. Summaries of the more pertinent are as follows:

Sections of the pulmonary artery showed the tear extending through all layers including the pericardium (Fig. 2). The slight lateral dissection was between the fibers of the outer fifth of the media which were spread apart by the blood clot (Fig. 3). Small, typical atheromatous intimal plaques were present. The media contained irregular spaces filled with an acellular, homogeneous-staining

mucoid material which disrupted the normal pattern of elastic fibers, muscle and fibrous tissue (Fig. 4). The nuclei were absent in the tissues surrounding these spaces, and many fat droplets were present. The muscle was decreased in amount, the elastic fibers were farther apart, some thicker than normal, others frayed and fragmented. Fibrous tissue crowded the elastic fibers apart and replaced them in moderately large areas, especially near the adventitia. The adventitial and subpericardial tissue near the rupture was heavily infiltrated with red blood cells. Small areas of early thrombus formation showed marked fibroblastic proliferation with many round cells, plasma cells and scattered eosinophiles. The arteriolar walls of the adventitia and outer media were thickened by intimal proliferation so that the lumens were more than half occluded in some instances and the venules were distended with blood. There was slight round cell infiltration of the vasa vasorum. The picture suggested that the pathological process had been present in the outer wall of the pulmonary artery prior to the fatal rupture. Giemsa, acid-fast, and spirochete stains revealed no organisms.

A section through one of the larger intimal plaques in the pulmonary artery away from the line of rupture showed rather marked intimal thickening due to acellular hyalinized fibrous tissue with fatty degeneration, necrosis and cholesterin crystals extending into the media. The media was thinner, with fewer elastic fibers and more definite breaks in continuity beneath the plaque than in other areas (Fig. 5). The adventitia was normal except for marked congestion.

Sections of the liver typified varying degrees of "cardiac cirrhosis" ranging from dilatation and congestion of the central veins and sinusoids, with atrophy and apparent increase in central lobular fibrous tissue, to areas of marked fibrosis with obliteration of the lobules, leaving scars causing depressions in the liver capsule with intervening hypertrophic nodules of liver tissue. The periphery of the nodules was not invaded by cellular fibrous tissue as in the ordinary type of nodular cirrhosis.

The thyroid nodule was a well encapsulated, fetal type of adenoma. The stomach showed a benign fibrosing ulcer.

The lungs were normal except for slight edema and scattered areas of hemosiderin-laden phagocytes. There was no material increase in fibrous tissue. The walls of the arterioles were normal but

the intima of the larger arteries showed thickened fibrous plaques with fatty changes.

Anatomical Diagnoses: Chronic endocarditis with mitral and tricuspid stenosis and insufficiency; marked right sided cardiac hypertrophy and dilatation; slight dilatation of the pulmonary artery; moderate pulmonary sclerosis; fatal rupture of the pulmonary artery with hemopericardium; marked "cardiac cirrhosis"; generalized passive congestion; fetal adenoma of the thyroid; healing ulcer of the stomach; hypoplasia of the left kidney; old infarcts of both kidneys; melanosis coli; endocervicitis; fibromyoma of the uterus; and healed apical pulmonary tuberculosis.

Discussion of Literature

The etiology of spontaneous ruptures and dissecting aneurysms of large vessels has long been in dispute, and the theories as to the mechanism of their development are varied. Since these conditions are practically limited to the aorta, the theories have evolved about their occurrence in this vessel. A typical dissecting aneurysm begins in the ascending portion of the aorta with a transverse tear through the intima and outer two-thirds of the media with blood dissecting between the medial layers. The splitting may involve the entire aorta and extend into the iliac arteries. Commonly the blood ruptures externally into the pericardium, pleura or mediastinum with death of the patient, but it may rupture back into the lumen, or may heal, and the patient may live normally for many years.[16] Spontaneous rupture is a term best reserved to describe those cases of sudden death due to the bursting of a large vessel with very little gross evidence of the cause of the fresh tear and but slight dissection. The fundamental processes of these two conditions are probably the same.

Laennec[17] reported primary rupture of an atheromatous intima as the cause of dissection in the aorta. Peacock[18] was unable to produce the lesion in normal aortas by increased pressure in the lumen but dissection occurred when the intima was damaged transversely to the long axis of the vessel. In 1852 von Rokitansky[19] emphasized degenerative changes in the media and since that date many writers have favored medial changes with high blood pressure and primary rupture of the intima as essential factors. The medial damage has varied from inflammatory changes with destruction of the

elastic fibers to minute hemorrhages, tears and muscular atrophy. Lifvendahl [20] reported three cases of spontaneous rupture of the aorta with uniform changes consisting of an intimal tear at the base of the aorta, high blood pressure, renal arteriosclerosis and syphilitic mesaortitis. He emphasized the absence of extensive gross changes in the aorta but found considerable microscopic destruction. Tyson [12] in 1931 reviewed the many theories and in reporting 5 cases, among which were 3 with intact intimal lining of the aorta, agreed that dissecting aneurysm is dependent upon degenerative changes of the medial coat, probably due to obliteration of a large number of vasa vasorum from arteriosclerosis or a low grade inflammatory process. He postulated the formation of a hematoma, formed by rupture of one or more vasa vasorum, which split apart the medial fibers with secondary intimal tears.

The aortic and pulmonary trunks are histologically quite similar, but it is a fact that atheroma, syphilis and aneurysm are common in the aorta and rare in the pulmonary artery. Possibly the natural differences in blood pressure and the variations in carbon dioxide and oxygen content of the blood in each are pertinent.

Discussion of Cases

Duffield [13] made no suggestion as to the etiology in his case of dissecting aneurysm of the pulmonary artery. This was a 50 year old female with pulmonary and tricuspid insufficiency, right heart dilatation and hypertrophy, dissecting aneurysm of the right pulmonary artery the size of a "duck's egg," containing fibrinous clot, aneurysmal dilatation of the left pulmonary artery without dissection, advanced atheromatous degeneration and hemopericardium.

The case reported by Durno and Brown [14] was that of a 33 year old male who died in his sleep. The pulmonary artery with its two main branches and a patent ductus arteriosus were uniformly dilated, the walls showing large plaques of atheroma. At the bifurcation of the pulmonary artery the intima and media were torn through, forming a small dissecting aneurysm which had burst into the pericardium. The right ventricle was markedly hypertrophied. They explained the etiology by atheroma and high blood pressure caused by the patent ductus arteriosus.

The mechanism of rupture in our case can only be conjectured. The tear must have caused almost instantaneous death from heart

tamponade. The sharp edges of the tear and the slight lateral dissection indicated a fresh rupture which must have been the result of high pulmonary arterial pressure and weakness of the vessel wall. The etiology of the medial and adventitial changes may have been the partial obliteration of the vasa vasorum, either by localized arteriosclerosis or the inflammatory process responsible for the valvular stenoses, with the resultant degeneration of the wall of the pulmonary artery as seen in the microscopic sections. Along the line of the medial adventitial junction small areas of early thrombus formation with marked fibroblastic proliferation and round cells suggested that hemorrhages had occurred there prior to the fatal rupture. Tyson's theory of hematoma formation before the rupture of the inner wall may have played some slight part in this picture.

The marked tricuspid stenosis with the resultant hypertrophy and dilatation of the right atrium and vena cava undoubtedly gave origin to the large pulsating liver observed several years previous to the patient's death, and also to the smaller, firm nodular liver found at autopsy. In gross and histologically, the liver showed a marked "cardiac cirrhosis" type V of Lambert and Allison.[21]

These 3 cases are similar in that all showed dilatation of the pulmonary trunk with atheroma, hypertrophy of the right side of the heart, and fatal hemorrhage into the pericardium through rupture of the pulmonary artery. No microscopic examinations were reported in the first 2 cases.

Clinical and Pathological Correlations

King [22] first noted that tricuspid insufficiency due to distention of the right ventricle in mitral stenosis led to a decrease in pulmonary congestion by the reflux into the atrium and systemic veins. Tricuspid stenosis obviously has a similar or even more marked effect on decreasing the blood flow into the lungs. This probably explains the absence of orthopnea in the patient in spite of the very high systemic venous pressure and also why she was able to live 16 years after her first attack of heart failure with such severe valve lesions. In this case the clinicians failed to recognize the tricuspid lesion from its effects on the circulation, even though the position of the diastolic murmur suggested such a lesion to one examiner. It should be reemphasized that tricuspid injury must be considered in a patient with chronic endocarditis who has intense cyanosis or venous en-

gorgement without orthopnea and with relatively little dyspnea when at rest. In such a case the absence of vigorous pulsation of neck veins points to a physiological stenosis, not insufficiency. Although the clinical result of the tricuspid lesion is to diminish pulmonary inflow, in this case there was definite pulmonary hypertension as a result of the mitral lesion. But the gross changes in the pulmonary artery were slight and the sudden separation of the intima, in this case as in many cases in which the aorta is involved, cannot be explained merely by the chronic lesions of the artery which precede it, or by pulmonary hypertension. Just as thousands of aortas are subjected to severer stress or have more advanced degenerative lesions than the one that develops dissecting intramural lesions, so also hundreds of pulmonary arteries, subjected to higher pressure and higher pulse pressures by patent ductus or pulmonic fibrosis, withstand these stresses even after marked atherosis has developed.

The areas of the valve openings, in this case, were for the tricuspid 1.8 cm., pulmonic 3.9, mitral 3.9, aortic 4.3. The rigid mitral and tricuspid valves obviously must have been open during systole. As the pressure in the large arterial trunks is higher than in the auricles, a mitral opening nearly as large as the aorta must have permitted about as much blood to flow back into the auricle and pulmonary veins as entered the aorta at each beat. Curiously enough, there was no systolic apical murmur, which indicates that one cannot gauge mitral insufficiency by the heart sounds, or recognize pure stenotic lesions by the faintness of the systolic as contrasted with the diastolic apical murmur. Even with some pulmonic hypertension, the pulmonary pressure is much lower than the aortic, and with systolic apertures in the auriculo-ventricular valves bearing the same relation to arterial openings, reflux into the right auricle will be less than on the left. Here the tricuspid valve was less than half as large as the pulmonic, so that systolic reflux was less than half as great into the right auricles as into the left, while the impediment to diastolic inflow was twice as great. The anatomical findings point, therefore, to a tricuspid lesion which was physiologically stenotic in its effects.

Summary and Conclusions

1. A case of sudden death from a spontaneous rupture of the pulmonary trunk in a female 44 years of age who had suffered periods of marked cardiac decompensation for 16 years is presented.

2. Gross changes in the pulmonary trunk were inadequate to explain the rupture, but microscopic alterations of a degenerative nature were rather marked. These in combination with increased pulmonary arterial pressure must be considered as the cause of the rupture.

3. The tricuspid stenosis probably explains the absence of orthopnea, and also the fact that the patient lived so many years in spite of chronic heart failure and cyanosis.

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DESCRIPTION OF PLATES

PLATE 131

FIG. 1. Photograph of the pulmonary trunk showing the longitudinal rupture extending from the valve into the left branch.

FIG. 2. Photomicrograph of the tear extending through all layers of the pulmonary trunk and pericardium. Elastic tissue stain. × 10.

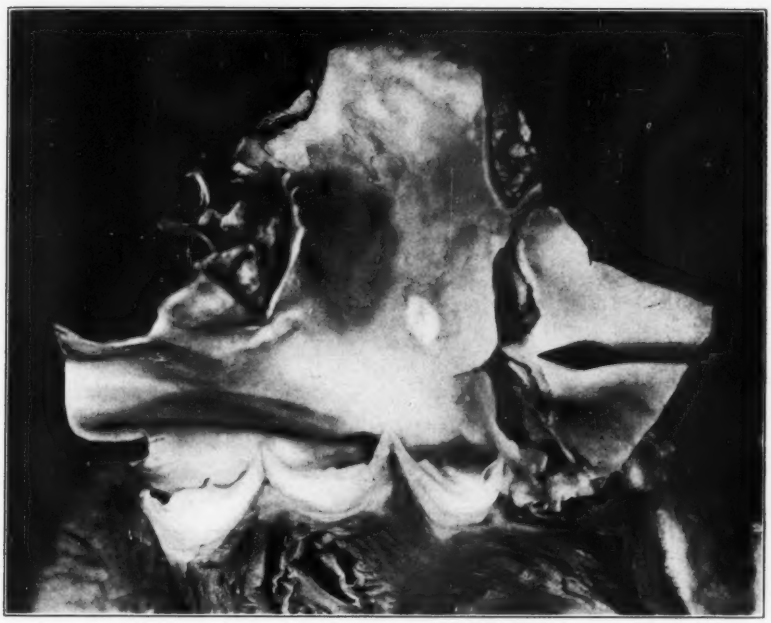

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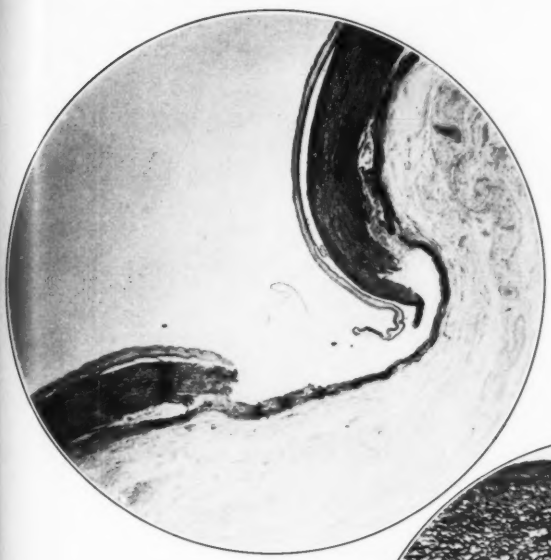
PLATE 132

FIG. 3. Photomicrograph of the tear through the intima and inner four-fifths of the media with lateral dissection. Elastic tissue stain. × 10.

FIG. 4. Photomicrograph showing irregular spaces filled with acellular mucoid material which disrupts the normal pattern of the media. Elastic tissue stain. × 80.

FIG. 5. Photomicrograph showing marked intimal thickening with thinning and breaks in the continuity of the media of the pulmonary trunk. Elastic tissue stain. × 80.



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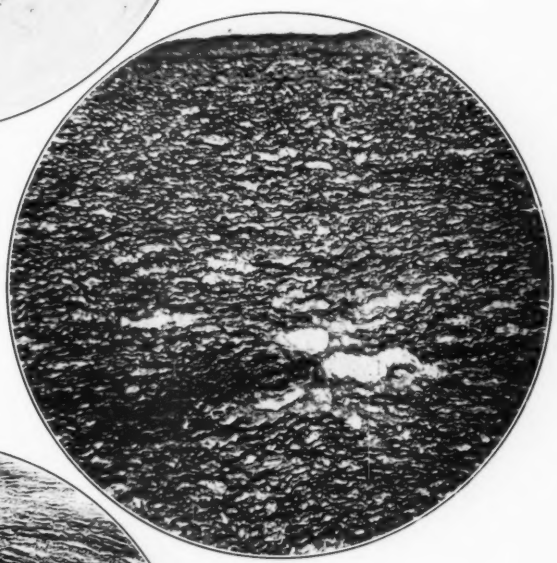

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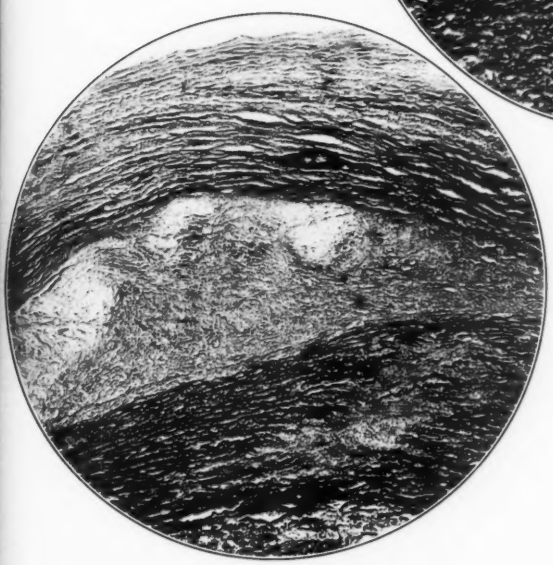

5

NEUROFIBROMA OF THE PHARYNX ASSOCIATED WITH VON RECKLINGHAUSEN'S DISEASE *

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Von Recklinghausen's disease, frequently referred to as neurofibromatosis, is characterized by multiple tumors of the nerves, mollusk-like skin tumors, pigmentation of the skin and elephantoid thickenings, isolated or in groups. The disease picture is frequently associated with other malformations and anomalies such as epispadias, cryptorchidism, uterus bicornis, polythelia, malformation of the kidneys, bones or muscles, and dwarfism or infantilism.[1]

According to Ewing [2] the cause of neurofibromatosis is not known, but it is assumed that such lesions are the result of a congenital malformation of the ectoderm, which under any one of a great variety of exciting causes may slowly or rapidly develop one or more of the manifestations of the disease. The work of Hoekstra [3] on the inheritance of this disease supports the idea of a dysontogenetic origin.

Primary pharyngeal neurofibromas developing either as a manifestation of von Recklinghausen's disease or as solitary tumors are extremely rare. Primary tumors of the oral cavity or pharynx arising from the nervous system or containing nerve elements are also rare. Neurofibromas developing about the face and neck most frequently occur in association with generalized neurofibromatosis and may secondarily bulge into the oral cavity.

Fibrous tumors are not common in the oropharynx and are to be distinguished from those in the nasopharynx. Those in the oropharynx are usually described as simple fibromas but they are probably never pure fibromas, the fibrous tissue usually being mingled with fat cells in variable amounts.[4]

New and Childrey [5] made a study of 357 cases of tumors of the tonsil and pharynx observed in the Mayo Clinic in a period of 14 years, from 1917 to 1930 inclusive. In this series no mention is made of neurofibroma, yet, their series contains 2 fibromas, 1 fibromyxoma, 2 fibrosarcomas and 1 fibromyxosarcoma.

The term neurofibroma is a descriptive term and seems to be ap-

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plicable to the tumor nodules found in von Recklinghausen's disease. Because of the varying mixture of nerve fibers with connective tissue the term neurofibroma is descriptive in the sense that it is a fibroma on and in a nerve, the fibers of which contribute toward the formation of the tumor. In retaining for the tumors of von Recklinghausen's disease the name neurofibroma, the term must be understood to signify a tumor that contains both nerve fibers and connective tissue. It is not a new growth of nerve tissue, although there are nerve fibers and apparently new nerve collaterals running in it. It is not a simple fibroma but is a fibrous connective tissue reaction that is a part of a more general process.

A pure neurofibroma, as seen in von Recklinghausen's disease, is in one sense not a neoplasm at all.[6] There are wandering nerve fibers derived from the involved nerve and a surrounding tangle of reactionary connective tissue which is a magnification of the widespread pathological alteration of the nerves in the system disease. Confusion arises from the fact that at times within these neurofibromas true neoplasms such as perineurial fibroblastoma may appear and may grow so large as to displace most of the neurofibroma tissue to the periphery. In von Recklinghausen's disease, however, nerve fibers will be found to enter each tumor with few exceptions, while in solitary perineurial fibroblastomas the comparatively normal nerve is invariably applied to the capsule of the tumor without penetrating it.

Grossly a neurofibroma is usually attached to a nerve. It is most frequently somewhat rounded and nodular in shape, firm in consistence and, on section, white and often almost translucent.

The histological picture is characterized by palisading and parallelism of the nuclei and a tendency to form nuclear eddies and streams. The nuclei are usually elongated and irregular. They may be large, however, and contain a condensation of chromatin which resembles to some extent the nucleoli of nerve cells, especially in degenerating areas.

Some authors attempt to differentiate perineurial fibroblastoma from true neurofibroma in that the former does not show nerve fibers. The nerve or nerve root to which the tumor is attached may be found at the periphery of the tumor running on or in the capsule, and at times a spiral coat ganglion may be dragged out by the nerve root and flattened over such a fibroblastoma.

A few cases of tumors associated with nerves located in the pharynx and adjacent structures have been reported in the literature. Spiess [7] reported what was apparently the first case of ganglioma arising in this location. This tumor arose in the subglottic region of the larynx. The upper part was visible between the vocal cords. Microscopically the tumor was composed of nerve tissue — a fibrous basis with numerous ganglion cells which had all the staining properties and characteristics of nerve cells. It is the opinion of the writer that gangliomas do not have the same histogenetic origin as neurofibromas and should not be classed with the type of tumor under consideration.

Colledge [8] in 1930 reported a case of a neurofibroma associated with von Recklinghausen's disease in a woman 44 years of age. The tumor presented itself as a firm swelling occupying the right aryepiglottic fold and pyriform fossa and entirely concealing the right vocal cord. It could not be palpated externally. Microscopically it proved to be a pure fibroma. One can not say definitely that there is any relation of this tumor to the generalized neurofibromatosis.

Forbes [9] in 1925 reported a case of plexiform neuroma (ganglion neuroma) associated with von Recklinghausen's disease in a girl 14 years of age. Oral examination showed a large, irregularly smooth mass occupying practically half of the faucial pharynx and involving the right tonsillar pillar. The left half of the pharynx was not involved. Grossly the specimen consisted of nine pieces of tissue; the largest measured 30 by 23 by 8 mm., and the smallest 10 by 5 by 4 mm. All pieces were irregular in shape, pale pink in color, semisoft in consistence for the most part and loose in structure with coarse, papillary projections on the free surface. On section there were white and translucent streaks throughout, suggesting fine, twisted nerve trunks. Meeker,[10] who reported the case from a pathological viewpoint, was unable to find any record of another similar tumor involving the pharyngeal mucous membrane. She considered this a true neuroma, a manifestation of von Recklinghausen's so-called neurofibromatosis.

Askanazy [11] reported a solitary nerve tumor of the post-pharyngeal wall which he, after ten years of study, finally designated as a "neurinoma Verocay," the modern designation of many of the tumors of von Recklinghausen's neurofibromatosis.

Figi [12] reported a solitary neurofibroma of the pharynx in a woman

61 years of age. This case is unique; I am unable to find a report of another similar one. Physical examination revealed extreme prominence of the left tonsil. It was firm and bulged almost to the median line of the pharynx, apparently because of the marked tonsillar enlargement. There was a palpable mass high in the left cervical region. At operation the tonsils were found to be uniform in size, the bulging on the left being due to a large, firm, somewhat irregular mass situated externally to the aponeurosis. Upon removal of the tumor it was found to be encapsulated with projections on its surface extending laterally and inferiorly. It was firm in consistence and measured approximately 4.5 by 6 by 8 cm., and on section was yellowish in color. Microscopically the tumor was reported to be a degenerating neurofibroma. Through a communication with Dr. Gorden B. New [13] I understand that he has recently removed a neurofibroma similar in location, size and structure from a young girl.

Suchanek,[14] Holmgren [15] and Vail [16] each reported a case of Schwannoma of the larynx, but no mention was made as to the involvement of the pharynx.

The writer is reporting a case of neurofibroma of the pharynx associated with von Recklinghausen's disease which is similar in type and location to the ones reported by Forbes [9] and Askanazy.[11] The case reported by Figi [12] was a solitary neurofibroma of the pharynx without the usual skin manifestations of von Recklinghausen's disease.

Report of Case

Clinical History: A white woman, 44 years of age, was admitted to the Paterson General Hospital on April 25, 1934. She complained of a lump in her throat and progressive difficulty in swallowing and breathing for the past few months. Family and past history were not remarkable.

Physical examination revealed a growth in the pharynx, somewhat elliptical in shape, measuring about 8 by 10 cm. It occupied the left half of the pharynx and extended from the eustachian tube above to the epiglottis below. The tumor bulged anteriorly and obstructed a view of the larynx.

Over the chest, abdomen, back and extremities were many soft and firm, pedunculated and sessile tumor masses, some measuring as much as 2 by 1 by 1 cm. Associated with the tumor masses were pigmented areas of the skin varying in size from 1 to 2 cm. (Fig. 1).

On April 26, 1934, under general anesthesia, a firm tumor mass apparently fixed to the side and back of the pharynx was removed in two pieces. The larger piece measured 7.5 by 6 by 2.5 cm., the smaller 4 by 2 by 4 cm.

Pathological Report: (No. 34–380). Grossly the specimen consists of seven pieces of firm tissue, weighing together 50 gm. The largest piece measures 5 by 2.5 by 2 cm. All pieces are firm in consistence and, on section, white in color with a smooth cut surface. The outer surface of the larger pieces is nodular, irregular and covered with a dense fibrous capsule. Microscopically the characteristic histological features are the palisade-like and parallel arrangement of the elongated nuclei. The nuclei also tend to be arranged in streams, eddies and whirls. Running parallel to the long axis of the nuclei are long parallel fibers of collagen. In some areas the tumor is rather cellular, the cells are a little larger and some of the nuclei are hyperchromatic. Very rarely a mitotic figure is found (Fig. 2).

Diagnosis: Neurofibroma with an occasional mitotic figure.

On April 30, 1934, two of the tumor masses were removed from the chest.

Pathological Report: The specimen consists of two pieces of tissue. The larger is 1.5 by 1 by 0.4 cm. and is covered on one surface with skin. On the skin surface is a slightly elevated, soft tumor mass 1 cm. in diameter. The second piece is a pedunculated, firmer tumor mass measuring 0.4 by 0.4 by 0.2 cm.

Diagnosis: Neurofibromatosis.

The patient made an uneventful recovery and showed no signs of recurrence of the pharyngeal tumor at the end of 12 months.

SUMMARY

1. A report of the successful removal of a neurofibroma weighing 50 gm. from the pharynx of a woman 44 years of age who presented a typical picture of von Recklinghausen's disease is made.

2. The cause and anatomical structure of neurofibromatosis, as found in von Recklinghausen's disease, is discussed.

3. Only 3 other cases of neurofibroma of the pharynx were found in the literature.

NOTE: I am indebted to Dr. N. P. Lobsenz for his permission to report this case and for his assistance in obtaining the clinical data.

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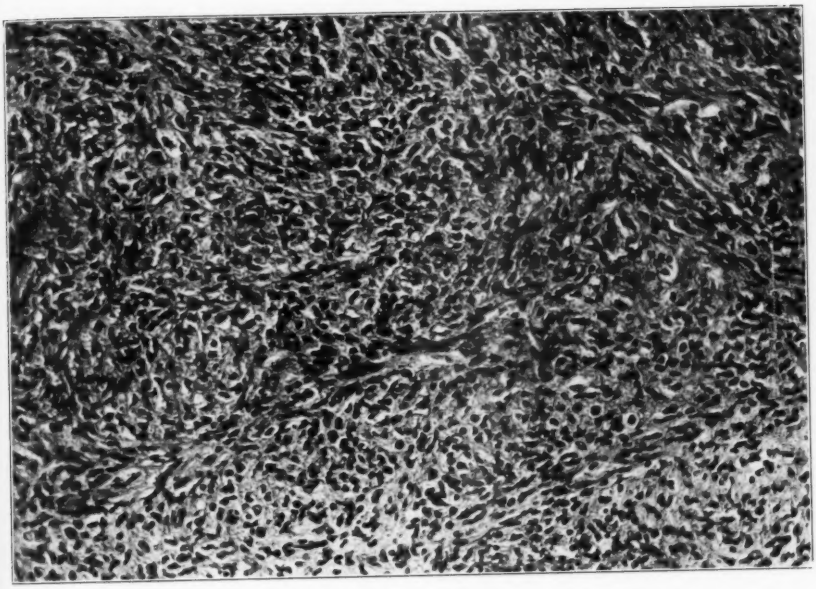
DESCRIPTION OF PLATE

PLATE 133

FIG. 1. Photograph showing neurofibromas associated with areas of pigmentation (von Recklinghausen's disease).

FIG. 2. Photomicrograph showing the most cellular area of the neurofibroma from the pharynx. × 120.



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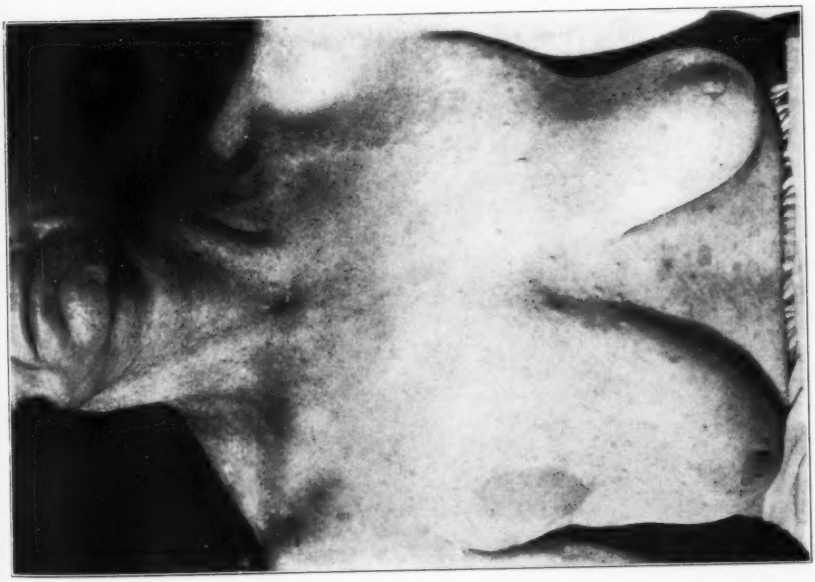

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A TECHNIQUE FOR DEMONSTRATING THE PERIVASCULAR NERVES OF THE PIA MATER AND CENTRAL NERVOUS SYSTEM *

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(From the Montreal Neurological Institute, Montreal, Canada)

In the course of a study of the nerves of intracranial blood vessels it was found that the Cajal reduced silver method and the methods employed by Stöhr and by Bielschowsky would at times demonstrate vascular nerves on the pial and dural vessels. But no such nerves could be demonstrated on intracerebral vessels. The method of Gros-Bielschowsky likewise stained nerves on all but the intracerebral vessels until it was modified as will be described below. It was only due to the persistance and versatility of our technician Edward Dockrill that a reliable method was at last devised which serves to demonstrate nerve fibers both on pial and intracerebral blood vessels.

The addition of acid to the formaldehyde fixative is the most important new element in the procedure. Different acids may be used but the best results were obtained with a combination of formalin and citric acid, which we have called Dockrill's fixative. Thick sections may be made but best results were obtained when the whole vessel dissected free from the brain was carried through the various solutions on a glass rod.

The routine procedure is as follows:

1. Wash out blood from the material to be used by perfusion of saline through the blood vessels, or by rinsing uninjected tissue in saline.

2. Fix, preferably by injection, with 10.5 per cent citric acid (powder is more readily soluble but crystals may be used) in 20 per cent commercial formalin (neutrality of the formalin used is not important). If injection is not possible leave the material 2 or 3 days in fixative before staining. Otherwise stain shortly after injection.

3. Prepare tissue by dissecting out blood vessels under a dissecting microscope. The pia is cut and turned slightly backwards so as

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to expose the vessel entering at right angles to the brain. Brain tissue is then carefully pushed away from around the vessel until a sufficient length is obtained when the vessel is cut and lifted out. Intracerebral vessels of the anterior perforated space and large branches of the middle cerebral artery, such as the lenticular striate, are the most readily dissected.

4. Wash blood vessels or sections in 2 changes of distilled water and place them in a 20 per cent aqueous solution of silver nitrate for 2 hours.

5. Pass material through 4 changes of 20 per cent formalin. Use Petri dishes for this and have about 100 cc. of solution in each dish. (It is a good plan to have both the formalin and the subsequent materials laid out ready for use before beginning this step; see schema below.)

6. Pass directly from 20 per cent formalin to ammoniacal silver nitrate, prepared by adding concentrated ammonia (28 per cent) drop by drop, to a 20 per cent solution of silver nitrate. A precipitate forms which redissolves on addition of more ammonia. Add about 3 drops in excess. If the material turns black or becomes too dark, cautiously add more ammonia drop by drop until the concentration is right as seen by the tissue. Examine the vessel or section under the microscope for degree of staining. The nerves will be seen to "come up" slowly.

7. When the degree of staining is sufficient place for 1 or 2 minutes, according to thickness, in 20 per cent ammonia water.

8. Wash in distilled water acidified with 12 to 15 drops of glacial acetic acid. Any trace of alkalinity precipitates gold chloride from solution; that is the reason for the acidified water wash.

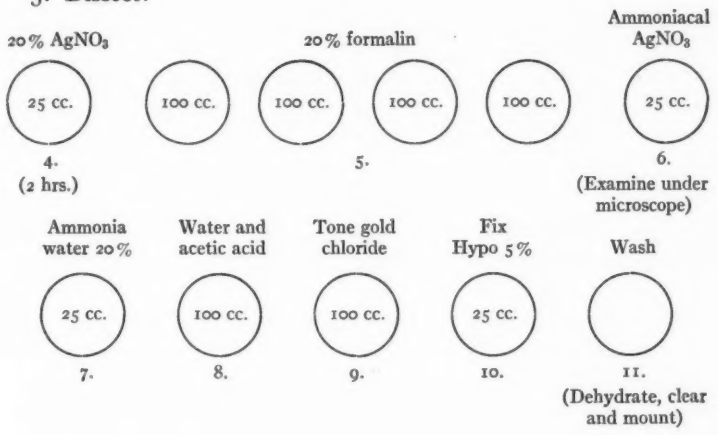
9. Tone in 0.2 per cent (1:500) yellow gold chloride for ½ to 1 hour.

10. Fix in 5 per cent sodium thiosulphate 15 minutes.

11. Wash in water. Dehydrate in 3 changes of 95 per cent alcohol and mount in Canada balsam from carbol-creosote-xylol mixture.

Summary

1. Wash.
2. Fix.
3. Dissect.



It often happens that insufficient staining of nerves is obtained because of precipitate forming before staining is complete, or because the material darkens too rapidly in the ammoniacal silver bath. Such material can be decolorized and then, after refixation, stained again. Two solutions are used for this, each kept in a separate bottle. The first is a 10 per cent solution of iodine in a 20 per cent aqueous solution of potassium iodide. The second, a 10 per cent aqueous solution of potassium cyanide, is added drop by drop to the iodine until it clears. When the stained vessel or section is placed in this it becomes almost instantly colorless. Wash in water and leave in fresh fixative for 2 or more days before restaining. This procedure will often "bring up" nerves that have previously failed to show and one may repeat the process as many as a dozen times on the same material. Care should be exercised in using it, however, as the solution liberates hydrocyanic acid fumes.

Preparations made as described above seem to last indefinitely as those now 5 years old have not changed. The nerves on the intracerebral vessels are continuous with those upon the pial vessels and nerve endings may be impregnated at times.[1, 2]

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PAPILLOMATOSIS PERITONEI *

ARTHUR H. WELLS, M.D.

(From the William Volker Laboratory of Research Hospital, Kansas City, Mo.)

A benign, branching villous papilloma, the surface cells of which are continuous with the mesothelium of the peritoneum, is of considerable rarity since we can find no record of a similar case in the available literature, nor have we found others familiar with the condition.

In the case to be reported the patient's medical history and death apparently have no relation to the peritoneal papillomas. However, a brief summary is presented.

A 70 year old judge had been in excellent health, never having suffered from any serious disease, but there gradually developed over a period of 1 year an increasing difficulty in voiding. The symptom progressed to complete urinary retention during the last 2 weeks of life. There was a moderately enlarged prostate but no other important physical abnormalities, except for an occasional extrasystole. The urine showed a cloud of albumin and many pus cells. Blood chemistry, Wassermann test and other routine laboratory procedures were negative. A suprapubic cystotomy was performed as the first stage of a prostatectomy and there was some attempt at repair of a urinary bladder diverticulum. The patient remained in apparently good condition throughout the operation. The pulse, respiration and blood pressure taken 4 hours postoperatively (5 minutes before death) were normal and the patient gave no evidence of impending sudden death.

The important findings at autopsy included a heart weighing 460 gm. with badly sclerosed coronary arteries but showing no myocardial scarring or evidence of myocardial degeneration. There were moderate congestion and edema of the lungs, congestion of liver, spleen and kidneys, obstructive benign prostatic hypertrophy, chronic prostatitis, acute cystitis with a large diverticulum in the fundus of the bladder and many small papillary tumors on otherwise normal peritoneal surfaces. The opened intestines contained

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no sign of neoplasm and no single primary source of the peritoneal lesions could be found.

The papillomas were scattered over the parietal peritoneal surfaces, being concentrated for the most part on the diaphragm, omentum and mesentery of the small intestine. They varied in size from slight irregularities, invisible to the unaided eye, to lesions nearly a centimeter in diameter and occurred singly or in small groups. Occasionally there was a small villous bridge between adjacent lesions. The smallest lesions were irregular elevations of the peritoneum with distinct increase in the size and number of mesothelial cells and with a change to cuboidal shape. A very slight increase in cellularity of the underlying connective tissue was noted. A few lesions were found as microscopic crypts below the surrounding peritoneal surface. Simple finger-like villi less than a millimeter in length were frequent. Microscopically they may have had many small buds covered by small cuboidal cells which showed no mitotic figures. In the largest lesions heavy villi branched from a common center at the top of a rather long and narrow pedicle. The main villi may have branched once or twice and small buds were located anywhere on these but more particularly on the smaller branches. The larger villi tended to be heavy and club-shaped and their mesothelium thin and flat, approaching the normal. The connective tissue was coarse and had comparatively few cells in the pedicle and proximal portions while the ends of villi showed fewer cells and very delicate fibrils. A few well formed blood vessels were found even in the small branches. Nerve fibers could not be demonstrated. The small numbers of lymphocytes present in a few of the older villi could hardly be considered the result of inflammation of bacterial origin.

An attempt was made to learn more of this tumor by direct correspondence. Dr. James Ewing's first impression was that the papillomas were epithelial implantations from some ruptured epithelial cyst. If no such primary tumor could be found, then by exclusion one must conclude that they were endothelial in origin and represent true fibro-endothelial papillomas. Except for multiple cystic tumors of the peritoneum, he had never seen anything like the case in question.

Dr. William Boyd felt that the condition was a papillomatosis of the peritoneum, but using the word merely to indicate a proliferative lesion of connective tissue cells projecting from the surface and not

necessarily an epithelial neoplasm, and although he had never seen or heard of papillary growths resulting from chronic irritation of the peritoneum, this was a possible etiological factor.

Dr. Arthur E. Hertzler believes the condition to be a primary, benign, wart-like growth of the peritoneum, the only one of its kind in his experience. A number of other pathologists and surgeons were of a similar opinion.

The tendency of certain embryonic derivatives of the peritoneum to form papillary tumors of a somewhat similar type as the above is a generally recognized phenomenon. It seems unusual that papillary tumors of peritoneum are not more frequently described.

Summary and Conclusions

An unusual and widespread villous type of benign peritoneal papilloma was an incidental finding at the autopsy of an elderly man dying 4 hours after the first stage operation for prostatectomy. The tumor is considered primary in the peritoneum and related to similar neoplasms in structures derived from the peritoneum.

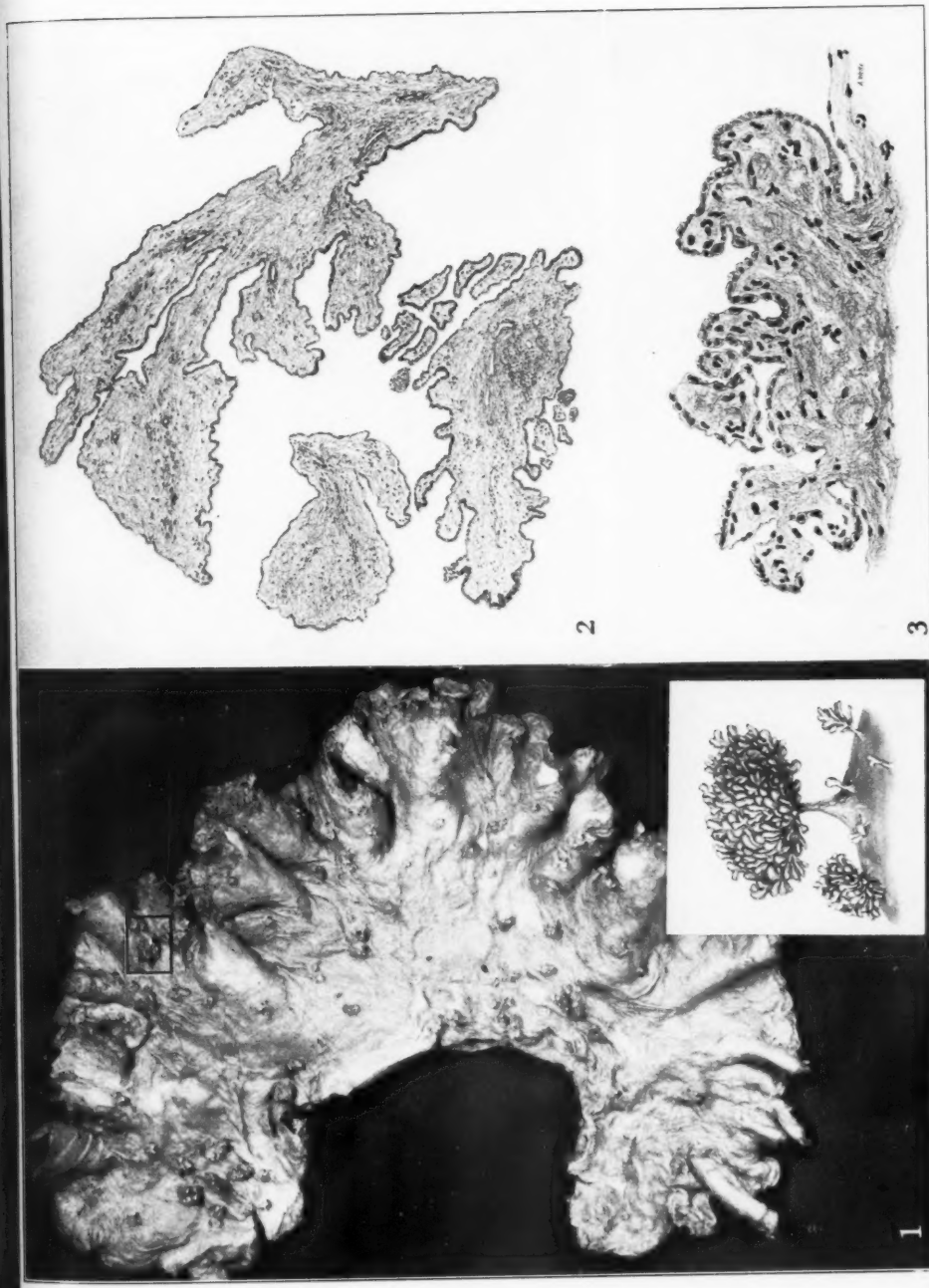
DESCRIPTION OF PLATE

PLATE 134

FIG. 1. Mesentery of small intestine covered with benign papillomas. (Inset) A group of lesions of varied sizes. × 5.

FIG. 2. Branching villi and "buds" from a large lesion. × 50.

FIG. 3. Early lesion showing relation to mesothelium. × 250.



Wells Papillomatosis Peritonei

INDEX OF SUBJECTS

INDEX OF SUBJECTS

A

B

* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at New York City, April 18 and 19, 1935.

C

D

E

K

L

M

N

O

P

R

S

T

U

V

INDEX OF AUTHORS

INDEX OF AUTHORS

A

B

C

* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at New York City, April 18 and 19, 1935.

D

E

F

G

H

J

K

L

M

N

O

P

R

S

Z